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RADIATION PRESERVATION OF FOODS PROGRAM

Proceedings of Eighth Contractors' Meeting

U. S. Army Natick Laboratories
Natick, Massachusetts
October 7-9, 1963

Sponsored by

U. S. ARMY NATICK LABORATORIES
U. S. ARMY MATERIEL COMMAND

and

ADVISORY BOARD ON MILITARY PERSONNEL SUPPLIES
(Committee on Radiation Sources)
(Committee on Radiation Preservation of Food)
Division of Engineering and Industrial Research
National Academy of Sciences—National Research Council

Edited by

Frank R. Fisher
and
Edward S. Josephson

Washington, D. C.
U. S. Department of Commerce, Office of Technical Services
1964

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FOREWORD

These proceedings of the eighth meeting of contractors of the U. S. Army Natick Laboratories Radiation Preservation of Foods Program record the formal presentations which encompass the status, objectives, and plans not only for the Army program, but also for the Atomic Energy Commission Radiation Preservation of Foods Program.

In contrast to previous contractors' meetings devoted to the Army program, this one was designed to afford the attendees an opportunity to obtain an over-all view of the Nation's Radiation Preservation of Foods Program and to exchange information on a formal and informal basis in order to accelerate the contributions to the science and technology of radiation preservation of foods.

J. Fred Oesterling
Deputy Scientific Director for Research
U. S. Army Natick Laboratories

CONTENTS

SESSION NO. 1

Dr. Edward S. Josephson, Chairman
U. S. Army Natick Laboratories
Natick, Massachusetts

WELCOME REMARKS

Brigadier General Merrill L. Tribe

Ladies and Gentlemen:

Welcome to the Natick Laboratories. The relatively long interval between the Seventh (June 6-8, 1961) and Eighth Contractors' Meetings should not be construed as a slowing down of the Radiation Preservation of Foods Program. Much progress has been made in the interim. Nevertheless, many objectives remain to be accomplished. You will hear of these matters during the next few days.

This assemblage is comprised of 1) past and present contractors of the Army and Atomic Energy Commission programs, and 2) those with a direct interest in preservation of foods by ionizing radiations, who are either actively working in the field or plan to be active in the future. These conferees come from industry, universities, research foundations, and governmental agencies. I wish you all a successful and stimulating exchange of information.

We at Natick are appreciative of the National Academy of Sciences—National Research Council, Advisory Board on Military Personnel Supplies, Committees on Radiation Sources and Radiation Preservation of Food's participation in the organization and conduct of this meeting. We particularly thank Drs. W. George Parks and Frank R. Fisher, Executive Director and Executive Secretary of the Advisory Board, for the myriad of things they have done to ensure a successful meeting.

The Atomic Energy Commission and Army Medical Service deserve special thanks for participating in this meeting. Their contribution assures the success of this meeting because the audience can get the complete picture of the varied efforts in this country focused on radiation preservation of foods.

I am sure you all know Dr. Adelbert W. Harvey of the Natick Laboratories. He has been with the Army Radiation Preservation of Foods Program in the early days and is retiring next week. We thank him for his many contributions to the program and wish him a comfortable and cheerful retirement.

THE ARMY RADIATION PRESERVATION OF FOODS PROGRAM, HISTORIC HIGHLIGHTS

Ferdinand P. Mehrlich

Ladies and Gentlemen:

I wish to take this opportunity to add my warmest welcome to those of General Tribe. This, the Eighth Meeting of the Contractors of the Radiation Preservation of Foods Program, is the first to be held at the U. S. Army Natick Laboratories. Previous meetings have been held at such diverse locations as Chicago, Illinois, University Park, Pennsylvania, Gatlinburg, Tennesse, Highland Park, Illinois. It is fitting at this meeting in our new home to review with you some of the highlights of the past, learning from the long and tortuous road we have traversed so that we can better attain our goals in the days ahead.

Interest in the use of ionizing radiations to preserve foods dates back to the turn of the century when early investigators such as the Curies, Becquerel, and other pioneers observed the lethal effects of these radiations on living tissue. Application of this phenomenon was primarily in the realm of medicine where the increased susceptibility of cancer cells over healthy cells to ionizing radiations served as the basis for treatment of certain types of cancer. The application of these radiations to destroy microorganisms which are responsible for food spoilage or food-borne disease remained largely a dream because of exorbitant cost and non-availability of ionizing radiations for the large tonnages of foods which would require processing. It was not until the end of World War II when radiation sources became more abundantly available and at a cost within the budgets of research organizations that serious consideration could be given to the use of these radiations for food preservation. The Army, with its worldwide commitments and planned operations under conditions of rapid movement and dispersal, required a type of ration consisting of non-perishables independent of refrigeration. Ionizing radiations offered great potential for preservation of highly acceptable nutritious foods suitable for incorporation into military rations. Accordingly, the Quartermaster Research and Development Division, Office of The Quartermaster General, and the Navy's Research and Development Division, Bureau of Supplies and Accounts, closely followed the pioneering work done by Proctor, deGraaff, and Fram, of MIT, and Brasch and Huber of the Electronized Chemical Corp. By the end of the decade of the 1940's the Defense Department was sufficiently encouraged to consider entering the field of food preservation employing ionizing radiations. The Atomic Energy Commission beginning about 1950 supported investigations in universities in the use of gamma rays for food preservation. Because of

the many problems involved in bringing the radiation process to commercialization, it was apparent that the U. S. Government would have to provide the major financial support. Accordingly, in 1953, the Quartermaster General requested the Committee on Foods of the Advisory Board on Quartermaster Research and Development of the National Academy of Sciences—National Research Council to review and make recommendations as to the desirability of the Quartermaster Corps' initiating a substantial research effort in this field. An ad hoc Subcommittee created by the Committee of Foods of the NAS-NRC recommended unanimously that such a program be initiated and stated, "No other new method of preservation of food is so far advanced, appears to offer greater possibility, or is as economically feasible as is the radiological sterilization method in its present state of development." As a result of this study the Defense Department allocated funds to the Army Quartermaster Corps in mid-1953 to initiate a program on the use of ionizing radiations to preserve foods and the QM Food and Container Institute in Chicago, Illinois was assigned the over-all responsibility for conduct of this research. The philosophy was expressed at the outset that the Army would carry the program over the technological hump as quickly as possible so that industry could continue further development leading to commercialization without a large financial risk concommitant with the pioneering nature of this research. By mutual agreement the food technology studies of the Atomic Energy Commission were assimilated with those of the Quartermaster Corps. Between 1954 and 1960, with the exception of a small amount of work by the U. S. Department of Agriculture, the Department of Army conducted the entire government effort. These studies were carried on both inhouse and through contracts with approximately 100 academic, institutional, and industrial organizations. It was recognized that the program as initiated by the Army was of interest to other governmental agencies. To tie together the multilateral facets of the program, an Interdepartmental Committee on Radiation Preservation of Foods was established in the spring of 1956. The objective of this committee was to provide broad guidance for the radiation preservation of foods, achieve a higher level of participation by government agencies and industry, and effect a rapid transition of the program from the Department of Defense to other government agencies and industry.

Paralleling Executive Department interest in the program was a concommitant Congressional interest manifested through its Joint Committee on Atomic Energy. This Committee has held several hearings on the Army's progress; the program has had the continuing warm support of this Committee. Of particular significance was the melding of the program into the President's "Atoms for Peace Program", which led to increased interest both in the United States and abroad.

Meetings of contractors and other interested parties, of which this is the eighth, have been held beginning in 1954. These meetings are intended to bring about the broadest possible dissemination of information, airing of problems, and discussions of possible solutions—all leading to advancing as rapidly as possible the state of the art of radiation preservation of foods.

Within two years after the Army became extensively involved in radiation research, results were sufficiently promising to encourage the Army to seek funds to construct a pilot production facility for processing a variety of irradiated foods. Industry responded to the Army's request to enter into a partnership in this enterprise by forming Irradiated Products, Inc., consisting of Armour and Co., Continental Can Co., Food Machinery and Chemical Co., and General Foods. A Radiation Planning Agency located in the Office of The Quartermaster General, Washington, D. C., was formed to draw plans for constructing and operating the U. S. Army Ionizing Radiation Center (USAIRC). A site at Sharpe General Depot, Stockton, California, was selected for this Center. Without belaboring the issue at length, you are familiar with the premature reports which circulated at that time of allegedly adverse effects observed in animals which had consumed irradiated foods as the major constituent of their diets. These so-called anomalies led to a realignment of the food radiation program in late 1959 and early 1960. Construction plans for the center at Stockton were suspended. As a result of an "agonizing reappraisal" of the program a 6-year Army Research Program concentrating on the use of high radiation doses for meats was drawn up and approved by all echelons in the Defense Department and by the Joint Committee on Atomic Energy, Congress of the United States. The Atomic Energy Commission re-entered the Food Preservation Program by assuming primary responsibility for low dose treatment of selected marine products and fruits. As a result of this realignment, the program today is on firmer footing than heretofore. And when the dust settled, the alleged hazards of consuming irradiated foods were interred through the outstanding scientific studies on wholesomeness conducted by the Army Medical Service and its contractors.

The spanking new Army Radiation Laboratory you visited this morning was authorized for construction as part of the revised 6-year program. At the Army's request, the Atomic Energy Commission agreed to construct the laboratory and selected Associated Nucleonics as the architect-engineer. The laboratory contains two radiation sources, a 1.3 megacurie cobalt-60 source and a 24 mev, 18 kw electron linear accelerator, as well as a food preparation area and a taste kitchen. This laboratory is unique in the literal sense because there is no other one like it where under one roof nuclear physicists, engineers, food technologists, and home economists work side by side to advance the state of the art. Ground for this laboratory was broken in May 1961. On 28 June 1962 construction was essentially completed and the laboratory was dedicated with appropriate ceremonies. With beneficial occupancy of the laboratory achieved in June 1962, responsibility for the Army's Radiation Preservation of Foods Program was transferred from the QM Food and Container Institute to the Natick Laboratories.

In closing, I would like to pay a tribute to all the individuals who stuck with the program through the trying periods and particularly to Mr. Abe Anellis, Miss Martha D. Driscoll, Dr. Adelbert W. Harvey, Dr. Fred Heiligman, Mr. Albert S. Henick, Major Sarah F. Niblack, Major Reuben Pomerantz, Mr. Morris Simon, and Dr. Ralph G. H. Siu. Their dedication and faith laid the groundwork for the achievements which succeeding speakers will cover.

THE ARMY RADIATION PRESERVATION OF FOODS PROGRAM PRESENT AND FUTURE

Edward S. Josephson and Morris Simon

June 1962 marks a convenient starting point for the subject of my discussion. It was during that month that the Army's Radiation Preservation of Foods Program began to be phased out at the Armed Forces Food and Container Institute in Chicago and that the build-up at Natick began. Also, during that month I completed a 10-month sabbatical at the Industrial College of the Armed Forces, Washington, D. C., and returned to Natick to take charge of the food irradiation program. It was also in June 1962 that the U. S. Army Radiation Laboratory was dedicated with appropriate ceremonies, although it was not until 5 November 1962 that the Atomic Energy Commission turned the building over to the Army.

During the period June to November 1962, we did have beneficial occupancy of the Radiation Laboratory and participated with the AEC and its contractors in a shakedown of the Laboratory and its equipment. Our license to operate the radiation sources was granted in September 1962 and became effective on November 5, the day the Army took possession of the building. We were, therefore, prepared to swing into almost immediate operation of the cobalt-60 facility on the day we formally took possession of the building. Today the Radiation Laboratory is completely equipped and operational with the exception of the linear accelerator upon which we are awaiting the installation of the conveyor. This event is scheduled for the end of December 1963. The laboratory, therefore, should be fully operational early in 1964. Construction of the Food Microbiology, Chemistry, and Nutrition Laboratories is now expected to be completed by the end of 1963. The Army's Radiation Preservation of Foods Program will then have superb laboratory facilities at Natick, supported by the capabilities of the Food Division as well as other divisions which make up the Natick Laboratories complex, including the Army Institute of Environmental Medicine.

If there is an Achilles heel in our inhouse capability, it is our difficulty in recruiting the nuclear physicist to head our Radiation Sources Branch. Fortunately, during the past year this gap was filled by Dr. Ari Brynjolfsson of the Danish Atomic Energy Commission. We ask your help in locating qualified candidates.

In July 1962 we submitted to the FDA the first Army petition for clearance of an irradiated food. This was for canned bacon sterilized with gamma rays from cobalt-60 at the 4.5 Mrad dose. It was our feeling, which I am

sure we shared with many of you, that the fate of the Army's Program rested on FDA action. FDA approval, announced on 8 February 1963, marked a turning point in the Army's program, climaxing 10 years of research. This clearance established an anchor for petitions for other irradiated food items to follow. It set the stage for approval of Prof. Lloyd Brownell's (University of Michigan) petition for disinfesting wheat at 50,000 rads (one-one hundredth of the radiation dose for bacon). FDA approval of this petition was announced on 21 August 1963. Other gamma-irradiated food petitions now pending before the FDA are: white potatoes (5000 - 10,000 rads) for sprout inhibition, submitted by the Army on 3 May 1963; oranges and lemons (75,000 - 200,000 rads) for inhibition of surface microorganisms, submitted jointly in behalf of the AEC and Army on 13 September 1963. The AEC recently has petitioned the FDA to broaden approvals for gamma radiation to cover all sealed sources with energies below 2.2 mev in order to expand the use of radioisotopes, give greater flexibility, and reduce processing costs.

Turning now to the use of electrons, the FDA on 23 August 1963 approved the General Electric Company's petition using Army data for the use of electron beams at the 5 mev level to sterilize canned bacon. The Army has a similar petition before the FDA (submitted on 25 July 1963) to permit the use of electrons at the 10 mev level.

Following is a list of products and the time frames in which the Army will petition for FDA approval for radiation processing at sterilizing doses:

Chicken Thighs & Breasts	FY 1965
Pork Loin	FY 1965
Ham	FY 1965-66
Shrimp	FY 1966
Beef	FY 1966

Also in preparation are joint AEC-Army Petitions for clearance of irradiated carrots, peaches, and nectarines.

It is now generally accepted that if a 5.6 Mrad dose is considered a safe dose (as a food additive), a lesser dose may automatically be considered safe. Therefore, wholesomeness data from the Army's high dose radiation program will be directly applicable to the AEC's pasteurization program. By Army-AEC agreement, joint petitions will be submitted to FDA wherein AEC will provide product development data and the Army will provide wholesomeness data. In this category are several species of lean fish, shrimp, clams, crabs, strawberries, and tomatoes, —all irradiated at pasteurizing doses.

We will now present a brie rundown of the status of the major tasks in the Army program. Detailed presentations on each of these tasks will be given by other speakers during this conference.

The task on Military Applications and Economic Analyses, currently unfunded, will be reactivated in FY 1965. The most recent data are embodied

in the Report[1] completed in August 1961 by the Operations Research Office of Johns Hopkins University. In fiscal year 1965, with the Army Radiation Laboratory's Co-60 and linear accelerator sources and the Bureau of Commercial Fisheries' AEC-funded Marine Products Development Irradiator in operation, we expect to obtain actual rather than projected pencil and paper figures.

The Army's Wholesomeness task on irradiated foods is directed by the Army Medical Service. On 1 March 1963, investigators engaged in this effort convened at the Walter Reed Army Medical Center, Washington, D. C., to assess the current status and to plan future research. The minutes of this meeting have appeared as Appendix 2 in the publication by the Joint Committee on Atomic Energy, Congress of the United States, 13 May 1963, entitled: Review of the Army Food Irradiation Program.[2]

In the course of these wholesomeness studies, which incidentally are the most comprehensive studies of this kind ever conducted anywhere, a number of anomalies were encountered. Statistical analyses of the data, however, indicated in some cases that these anomalies were not necessarily related to irradiation but may have been due to certain congenital factors in the test animals. For the most part, the adverse findings were due to vitamin deficiencies which could be corrected by addition of appropriate vitamins. Similar vitamin deficiencies have been reported on studies employing thermally processed foods. Laboratory studies have shown that radioprocessing is detrimental to some vitamins and that the vitamins affected are those which are also sensitive to heat treatment. Several years of testing on rats and mice by feeding irradiated foods and food components, injection of concentrates, or painting of concentrates on the skin, have shown no increased tendency toward carcinogenicity. Although additional work is still underway to resolve some of the anomalies, information generated under our program gives every indication that radiation processed foods are safe for consumption by humans.

On the question of induced radioactivity in irradiated foods, there is considerable controversy on the significance of the activity induced by electrons ranging in energy from 12 to 24 mev. Nevertheless, the increase in activity, though small, is measurable, and the present food additives amendment to the Food, Drug and Cosmetics Act does not permit the use of such foods for human consumption. We would like to suggest that wholesomeness research on selected foods treated with 12 to 24 mev electrons would make an outstanding contribution to the Radiation Preservation of Foods program. We believe that the Army Medical Service and its brilliant team of contract investigators is uniquely qualified to conduct such studies and obtain the needed information. The availability of such knowledge will be of immeasurable value in assessing the validity of the food additives amendment as it pertains to food irradiation.

In the task which we call "Pre- and Post-Irradiation Studies" we are primarily concerned with the palatibility characteristics of irradiated foods and are directing our efforts toward improving their odor, flavor, texture, and color. In this respect, we are looking into a variety of factors relating

to product and process, such as method of enzyme inactivation, the use of additives, irradiation at different temperatures, and application of new culinary techniques. Fundamental studies are being conducted simultaneously on the chemical and physical changes induced in irradiated foods and food components to obtain a better understanding of the nature and origin of these changes so that logical methods may be developed for their control. We do not expect quick and easy answers; continued progress will lead to significantly better second and third generation items.

The objective of our task in microbiology is to destroy microorganisms more effectively, and thereby lower the dose requirements for sterilization. A significant reduction in dose requirements will not only yield a better product, but will materially reduce processing costs and ease the radiation challenge to flexible packaging materials. In this endeavor, our main problem lies in overcoming the extreme resistance of Clostridium botulinum spores. Among the many approaches we have employed are the use of chemical additives (nitrates, nitrites, citrate, NaCl, etc.), elevated temperatures, and other forms of energy in combination with irradiation. Investigations are continuing on the physiology of spore germination and development in the hope that some means will be found for increasing the sensitivity of Clostridium botulinum to irradiation. Inoculated packs studies on bacon have not only demonstrated the safety of 4.5 Mrads, but have also suggested the safety of substantially lower doses. During the ensuing years we are planning to conduct inoculated packs on a number of meat and fish products to support our petitions to FDA for clearance of these products.

Our task on acceptance testing is highlighted by the resumption of tests with troops under garrison feeding situations at Fort Lee, Virginia. In June 1963, irradiated chicken, pork, and bacon were tested and found suitable for incorporation into military rations. Last week a second test was completed with oven fried, southern fried, and barbecued chicken but the data are not yet available to draw conclusions. It is planned to continue these tests at Fort Lee during the balance of this fiscal year and to incorporate selected sea food items emanating from the AEC Program.

Packaging studies have also been active and will continue to be active until suitable flexible packages can be obtained for radiation sterilized foods. Subsequent to the FDA approval of bacon, we have noted a marked upsurge in interest among a broad cross-section of fabricators of flexible packaging materials.

Our task on radiation services has become largely an inhouse task ever since the Radiation Laboratory became operational. In this connection, we are providing the radiation services this year to support the AEC's wholesomeness studies in addition to the SGO's and our Contractors. We do have many research problems in the field of dosimetry and in attempts to improve the operation and reliability of the linear accelerator.

Interest in the Army's Radiation Preservation of Foods Program is very high. The program was reviewed by the Joint Committee on Atomic Energy,

Congress of the United States (JCAE) on 5, 6 March 1962 in Washington, D. C.; and on 13 May 1963 at the U. S. Army Radiation Laboratory at Natick, Massachusetts. An International Conference on Radiation Research commemorating the operational availability of the U. S. Army Radiation Laboratory was held at Natick on 14-16 January 1963. The food radiation program has received extensive coverage in the press, radio, TV, magazines, and other media. Experimental meals with irradiated components have been served to the members of Congress, who are Army Reserve Officers, on 24 July 1962 in Washington and 14 June 1963 at Natick and will be served next week (October 15) in Washington. The JCAE was served an experimental lunch at the U. S. Army Radiation Laboratory on 13 May 1963. High ranking elected and appointed officials of the Federal, State, and Local Governments as well as military officers have toured the Radiation Laboratory and sampled irradiated foods. Visitors from all parts of the free world have been our guests.

Many of the problems that have required solution at the time of the Seventh Contractors' Meeting are still with us. We need to collect data to support our thesis that the safe sterilizing dose, at least for cured meats, is significantly less than 4.5 Mrads. We do not yet have a suitable flexible package. We have to conduct more research to find out how we can further improve our products. We are sure that these problems can be solved and that all major items of interest to the Army and to the AEC will in time be cleared by FDA. These facets are within our control, and a concerted effort will achieve success. As we solve these problems in our laboratories, we come face to face with the next problem: the commercialization of the radiation preservation of foods process so that irradiated foods, treated at both high and low dose, become abundantly available at a price the consumer will pay.

We consider the problem of commercialization of the process the most important one confronting us today. As an ancillary problem, there is the need to overcome unfounded apprehensions of laymen as to the wholesomeness of radiation processed foods. The two go together, because commercial interests are reluctant to invest if the customer is afraid to buy.

The benefits of irradiated foods to the military and civilian economies on a world-wide basis are so great, and the products we are developing are potentially so good, that we are confident that irradiated foods may appear in the market place within perhaps three to five years. Two and one-half years ago, at the time of the 7th Contractors' Meeting, the Army Program had just passed through the valley of the shadow of death. In June 1961 we had just passed a siege of stormy weather, and the future seemed uncertain to the faint of heart. Today, the climate is excellent.

We would like to conclude by quoting from Dr. Ralph Siu's presentation at the International Conference on Radiation Research, given at Natick on 14 January 1963:

> "These are the thoughts on science and man that go through the minds of those of us in the United States Army connected with

food irradiation as we look at the new Radiation Laboratory, and as we enter the final and rewarding phase of the program. There are still problems ahead. But the path looks clear. We are grateful for your timely participation and general assistance, and we look forward to an even more intensive collaboration as we jointly transform the experiments in the research laboratory into the reality of the dinner table."[3]

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SESSION NO. 2

THE AEC PROGRAM

Mr. Joseph Machurek, Chairman
U. S. Atomic Energy Commission
Washington, D. C.

PRODUCT DEVELOPMENT

Kevin G. Shea

Introduction

The U. S. Atomic Energy Commission's program for study of radiation pasteurization of food is described here. Spoilage by microorganisms is the main cause of rapid deterioration of perishable foods. By decreasing the number of bacteria, ionizing radiation at low dose levels postpones, although does not prevent, eventual multiplication of bacteria and thus inhibits damage to foods. Hence the irradiation process, in combination with refrigeration, is potentially highly advantageous in extending the marketing life of foods for shipment to remote areas. Related applications include disinfestation of grains, sprout inhibition of potatoes, and control of insects for quarantine purposes. The related U. S. Army radiation sterilization program is designed to develop a product that can be stored for months without refrigeration. Such a product is particularly suited to military logistics requirements, but may ultimately have civilian applications as well.

In the radiation pasteurization program, emphasis has been on a small number of selected food products that promise early technical feasibility and market acceptance. It is intended to define the processes for these products sufficiently to allow refinement and a final market test. Ultimately, the decision on success or failure will be made by industry in the market place.

History

The discovery of radioactivity by Becquerel in 1895 was followed closely by the idea of destroying microorganisms by this penetrating new force, with Prescott reporting[1] radiation effects on fungi in 1904. However, nuclear technology has only recently advanced to the point that large-scale use has become feasible. The results of an AEC-sponsored survey[2] published in 1951 indicated that a successful radiation process for food would improve the nutritional and public health aspects of the American diet; the AEC supported research in this field as early as 1950.

The first major effort in this field was instituted by the Quartermaster Corps of the U. S. Army in 1954, with a program to develop sterilized food with a reasonable shelf life at room temperature without refrigeration. They also supported some work on less-than-sterilizing doses.[3] The Office of the Surgeon General of the U. S. Army was, and still is, responsible for the

wholesomeness aspects of the Army program. In a continuing broad research and development effort instituted in universities, non-profit institutions, and private industry, more than one hundred foods have been subjected to microbiological, chemical, and physiological studies. Organoleptic characteristics, packaging, dosimetry, and related areas have also been investigated.

The Army plans included the construction of a pilot plant, now in operation, at the U. S. Army Radiation Laboratory. The laboratory is located at the U. S. Army Natick Laboratories, Natick, Massachusetts. This irradiation facility, built under AEC supervision, has a 1.1 megacurie cobalt source as well as an 18-kw variable-energy linear accelerator capable of providing electrons up to 24 mev. A large area is devoted to a preparation room and associated support facilities.

In March 1960 the United States Food Irradiation Program was realigned, and the AEC assumed the responsibility for the low-dose, or pasteurization, applications. This area suited the AEC objective of pioneering atomic energy developments that show special promise for industrial participation. The Division of Isotopes Development (DID) has the responsibility on source design and development, a field in which they have had long and varied experience—a crucial area needing investigation prior to the development of an efficient and economical process. The Division of Biology and Medicine has responsibility for the wholesomeness aspects of the program. In December 1960 the American Institute of Biological Sciences established a committee of scientists to consult with the Commission's Division of Biology and Medicine and the Division of Isotopes Development on irradiation processing of foods. Eight meetings have been held at six-month intervals, the first in August 1960. The comments and recommendations of the committee have been most helpful.

Close liaison is maintained by the AEC with the U. S. Army Natick Laboratories and with the Office of the Surgeon General. Some AEC personnel are members of the Interdepartmental Committee on Radiation Preservation of Foods, which provide channels of communication, through its constituent members, with the U. S. Department of Agriculture, Defense, and Interior; the Food and Drug Administration; and other interested Government Agencies. The importance of radiation processing of foods in the Atoms-for-Peace Program has encouraged cooperation and maintenance of close liaison with other countries interested in this activity. The AEC has participated in the Paris meetings of the Study Group on Food Irradiation of the Organization for Economic Cooperation and Development (OECD). The participating European nations have been kept fully aware of our progress and plans. We maintain contact with other international organizations such as the IAEA, WHO, FAO, and EURATOM.

USAEC Program

Significant progress has been made in the technology necessary to extend the shelf life of refrigerated fruits and marine products through irradiation, and a commercial process has been outlined for several food items. The

shelf life of these products has been extended up to five times normal, storage at lower temperatures giving maximum extension. Radiation doses required for product pasteurization are lower than originally anticipated. Based on previous process evaluations in this[4] and other programs the studies have been extended to include shipping and storage studies with a mobile Co^{60} unit located in California. Irradiation does not improve the quality of either fruit or marine products. The pre-irradiation condition and handling of the food are therefore important. Appendix 1 lists contractors doing work sponsored by DID. We have a total of 15 contractors. Our budget in 1963 was $1,516,000 including $600,000 for the Gloucester fish irradiator. In 1964 we hope for $1,975,000 including $675,000 for a research irradiator, a mobile fruit irradiator, and a grain irradiator.

Economics

Results now available from an economic analysis of the process as applied to fruit, being made by the USDA,[5] are promising. Food industry representatives feel that radiation processing will increase the production and marketing value of selected fresh fruits and vegetables but will not change the output and sales volume of canned, frozen, and other processed forms of the same commodity. The next phase of the study will determine the cost of the radiation process compared to existing treatments, and the probable impact of the method on the prices, suppliers, and market structures involved.

The Bureau of Commercial Fisheries of the U. S. Department of the Interior is conducting a two-phase study on which an educational program will be based. The first phase will determine the potential impact of radiation pasteurization of marine products on market suppliers, structures, and losses. Over 600 replies from distributors, processors, and others have been received to date. The second phase will be a highly qualitative study among consumers of fish and shellfish to determine the kind of educational program required for most successful marketing of irradiated fish. This will involve a study of attitudes held, information, misinformation, and psychological images related to fish in general and irradiated fish in particular.

Process

The program effort has been directed to the two classes of perishable foods (fish and fruit) selected as showing the most promise and later subjected to evaluation by Massachusetts Institute of Technology[6] and the Stanford Research Institute.[7] The fish originally included soft shell clams, haddock, shrimp, crab, flounder; recently certain fresh water fish have been added. The fruits originally included strawberries, citrus, tomatoes, peaches, and, more recently, cherries, figs, mangoes, and papayas.

Marine Products

Work done at the Gloucester, Massachusetts Bureau of Commercial Fisheries Laboratories shows that the refrigerated shelf life of fresh haddock and clams can be tripled with doses of 250,000 and 450,000 rads respectively. The quality of the fish prior to irradiation greatly affects the shelf life of the irradiated product, with fish one day old keeping for 30 days, but fish seven days old at the time of irradiation for only 11 additional days.

Work done at MIT indicates that a dose of 250,000 rads extends refrigerated storage life of haddock fillets 7-8 days at 43^{o}-46^{o}F and 14 days at 32^{o}-33^{o}F. For clams treated at the same level, the additional storage life is 16 days at 43^{o}-46^{o}F and 21 days at 32^{o}-33^{o}F.

At the Bureau of Commercial Fisheries Laboratories in Seattle, the life of Pacific crabmeat treated with 200,000 rads and held at 33^{o}F was extended from a normal seven days to 35 days. Petrale sole fillets treated with 400,000 rads responded similarly, their storage life being about twice as long at 33^{o}F as at 42^{o}F.

In addition to determining shelf life extension, other related work is supported. For example, the Seattle workers found that when radiation pasteurized samples became unacceptable due to bacterial spoilage (usually samples irradiated below 0.4 Mrad) good agreement was found in evaluating change in quality by total bacterial counts, yeasts and mold counts, total volatile base, and trimethylamine nitrogen content. In samples where a combination of higher irradiation dose and low storage temperature inhibited bacterial spoilage, other objective tests are needed to reflect the decrease in quality. The same workers found that air packed petrale sole fillets developed a rancid off-flavor that limited storage life.

Another interesting observation was that larger bacterial populations, approximately 100 times, are necessary to bring about flavor and odor changes in irradiated products than in non-irradiated products.

The relation of resistance of microorganisms to radiation is another area of work being supported. It is a fact that bacterial counts show extreme variation in irradiated fish or shellfish after 14-21 days of storage at 33^{o}F. These variations can range from no bacteria in one sample to as great as millions of bacteria per gram in another sample from the same lot of fish. In order to control those variations, it will ultimately be necessary to understand some of the factors that contribute to radiation resistance or radiation sensitivity.

It is well documented that microorganisms are more susceptible to ionizing radiation when they are in the logarithmic phase of growth. Previous research has shown that the lipid content of the bacterial cells is correlated with the growth rate of the bacteria. In other words, as the bacterial growth rate increases the cellular lipid content decreases. It can be therefore hypothesized that the cellular lipids act as a protective agent yielding a sparing effect

on other critical parts of the cell. The degree of unsaturation of fatty acids may also play an important role in bacterial resistance; as well as the role of the changed chemical composition of these dead fish, e.g., an increase in inosine and hypoxanthine.

Another area of continuing work involves the effect of irradiation on lipids of fish with the purpose of developing means of retarding undesirable changes. The rancidity that develops in stored fish is the result of oxidative reactions. Work is being done with docosahexanoic acid (C_{22}, 6 double bonds). Irradiation per se does not cause observable or measurable changes in this highly reactive fatty acid. However, we know that changes do go on at a higher rate in the irradiated product. Current work is concerned with the influence of metal ions on model systems.

At Louisiana State University, freshly caught shrimp, when properly handled, have had an iced storage life of 14-16 days. Treatment with 50,000 to 100,000 rads doubled this time. Melanosis or "black spot", a discoloration that occurs in shrimp from the action of phenol oxidases in shrimp, can be controlled by low doses of the order of 50,000 rads. Low dose radiation pasteurization does not offer much advantage for cooked shrimp. Holding the cooked product results in too much loss of flavor upon standing.

Louisiana State University is also doing work on the isolation and identification of the flavor components of shrimp and the changes which they undergo during irradiation. An attempt is being made to recombine these components to duplicate shrimp flavor. The most significant changes identified in the irradiated shrimp are the reduction of trimethylamine and ammonia and an increase in the methylamine content.

The workers have recently initiated work on oysters. Present results show that the storage life of low dose gamma irradiated oysters can be increased reasonably and that such a process is urgently needed by industry.

Fruits and Vegetables

Most of the work with fruit has been done at the University of California, Davis. Fruits that naturally have a short storage and market life will probably be of most interest for irradiation. Strawberries, sweet cherries, figs, oranges (Fig. 1), papayas, and pineapples, and perhaps peaches and nectarines, show the most promise to date. Preservation of strawberries and sweet cherries with dose levels of 200,000 to 250,000 rads has proved quite satisfactory.

The research has emphasized the feasibility and technology of the process such as permissible dose, storage and shelf life extension, quality effects, treatment variables, and evaluation of the factors involved in the various responses noted.

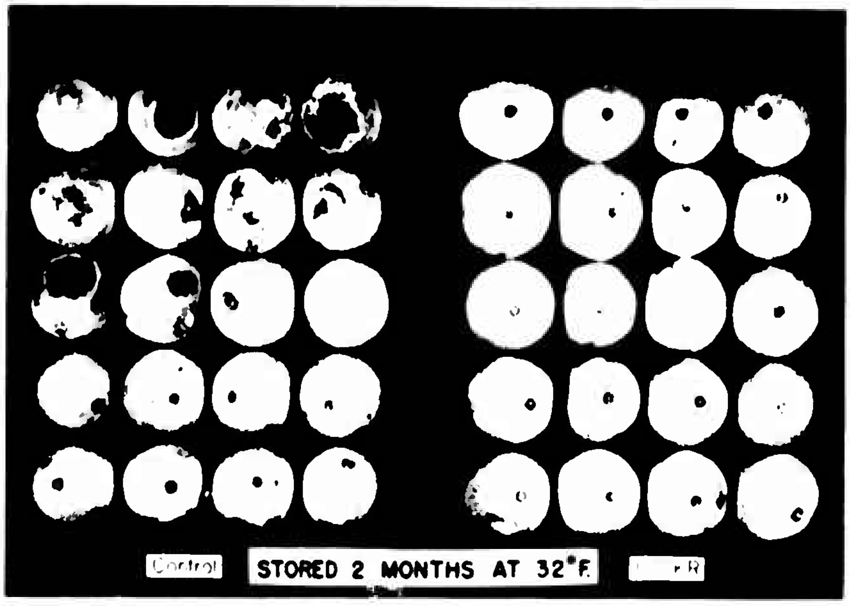

Figure 1. Oranges (Washington Navel Variety). Left: natural spoilage. Right: preserved by irradiation.

Lemon fruits show severe symptoms on injury if stored for a month after irradiation: air pockets develop in the fruits, the buttons are killed resulting in infection by Alternaria, and ascorbic acid concentration declines markedly. Oranges do not show these symptoms but do show some off-flavor development.

In strawberries, the amount of decayed fruit is reduced by a considerable amount at doses of 200 Krad and higher. The textural changes in response to radiation is less than in other species tested, flavor is not altered significantly, and other features of the fruit such as color and aroma were not considered objectionable by the taste panel. The ascorbic acid level in irradiated fruits was not altered by a significant amount.

Peaches and nectarines show textural changes at low doses. Nectarines are the more resistant, and some species of peaches resist this form of quality destruction. Respiration rates, ethylene production, and red color increase indicated an enhanced ripening on storage.

Results with grapes have been discouraging. The white variety in particular show severe textural damage.

In tomatoes, no ripening effect was noted at doses below 200 Krad, while doses between 200 and 400 Krad retarded ripening. The latter doses tend to cause undesirable effects on flavor. More work is needed.

Most recently, at the University of California, an extensive series of experiments on end point and dose survival analysis has been started for the common fruit pathogens such as Penicillium digitatum, P. Italicum, Botrytis cinerca, etc. Quantitative inoculations of the spore concentrations are made. The spore concentrations vary from 10 per 0.1 ml to 1,000,000 per 0.1 ml.

A plug of skin is pinched from the rind of an orange with a cork borer. The spore suspension is quantitatively piped into the wound, the wound is covered with scotch tape, the orange is irradiated and stored. It is becoming clear that the amount of radiation required to inhibit or inactivate a given pathogen depends very much on the number of spores in the lesion. For example, with very low numbers of spores it has been possible to keep oranges at 68°F for three or more weeks with little or no decay developing at doses as low as 100 Krad. With very high number of spores, it appears that doses in excess of 250 Krad may be required to give effective control. It begins to appear that radiation should be applied soon after the fruit is harvested and that radiation cannot be used to offset poor grower practices in connection with disease control in the orchard and in handling and storage procedures.

At the University of Florida, Gainesville, work has started on irradiation of tomatoes and citrus products. Michigan State University has initiated work on Mid-Western fruits, including cherries.

The University of Hawaii will investigate tropical fruits such as papayas, pineapples, and mangoes. Radiation treatment of Hawaiian fruits to meet U. S. mainland quarantine requirements is a logical application of the process, especially for control of the mango seed weevil. Equally important is investigation of shelf life extension by control of plant pathogens. Fruits such as avocados, for which a chemical treatment is now approved, might be better adapted to treatment by radiation. In addition, the USDA has initiated large scale storage and shipping studies of irradiated fruits and vegetables at their Research Laboratories in Fresno, California. They are using the modified AECL potato mobile irradiator as a source.

Irradiators

Conceptual designs for a grain irradiator,[8] an on-ship irradiator,[9] and a multipurpose irradiator (fruit disinfestation and shelf life extension) have been or will be completed shortly. The grain irradiator, to be located in Savannah, Georgia, will be constructed in the next calendar year. It will be operated by the USDA. Construction of the Marine Products Development Irradiator (MPDI) (Figs. 2 and 3), already started at Gloucester, Massachusetts, is scheduled for completion in late summer 1964. This $600,000 facility will operate on a near commercial scale, processing marine products at rates of up to 1 ton/hr. It will be operated as part of the research and development program conducted for the Commission by the Technological Laboratory of the Bureau of Commercial Fisheries, U. S. Department of the Interior.

The engineering design of a Co^{60} mobile irradiator has also been started. This irradiator will be used, particularly on the West Coast, for large-scale studies of fruit processing.

MPDI FLOOR PLAN

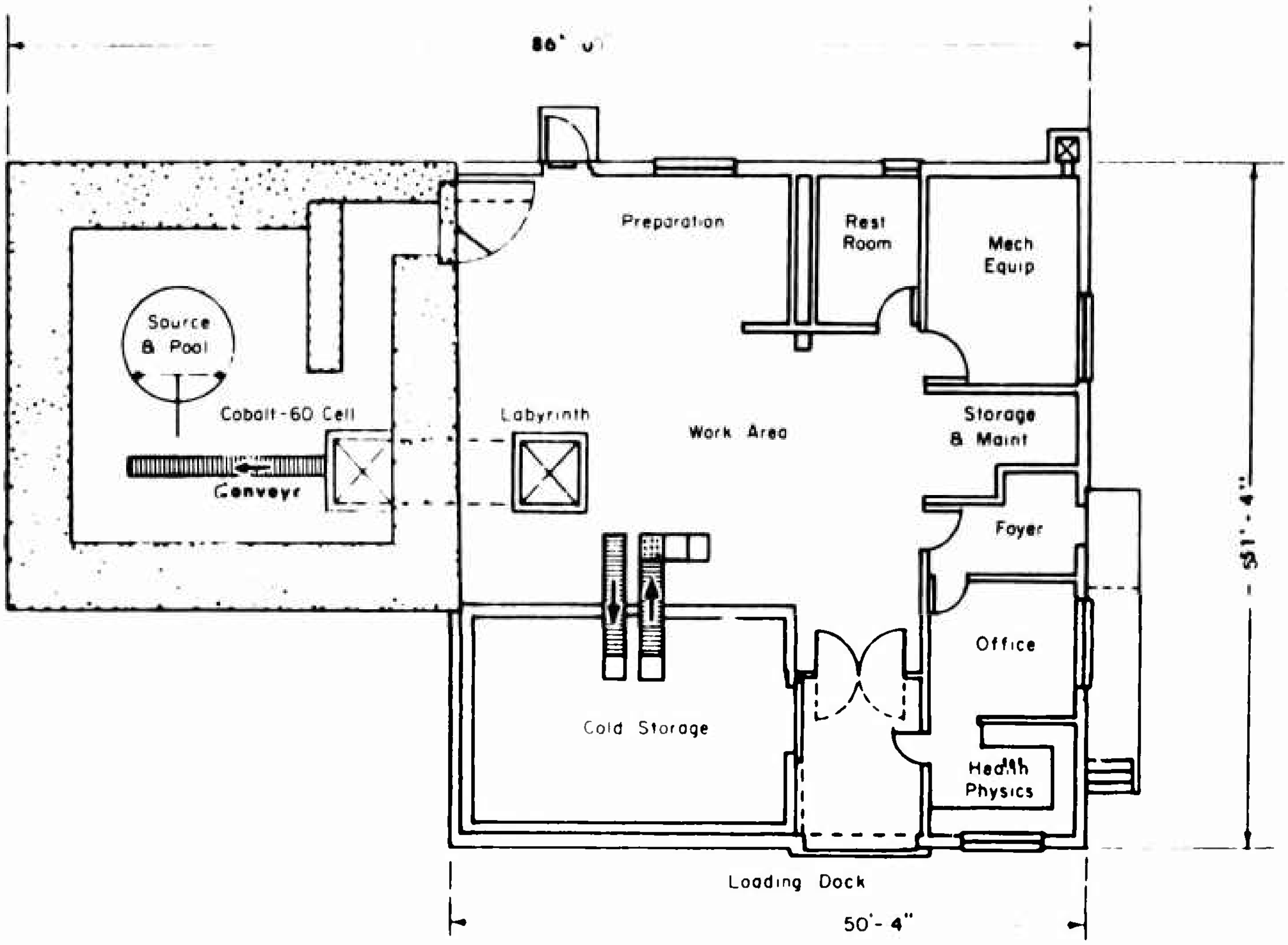

Figure 2. MPDI: Floor plan.

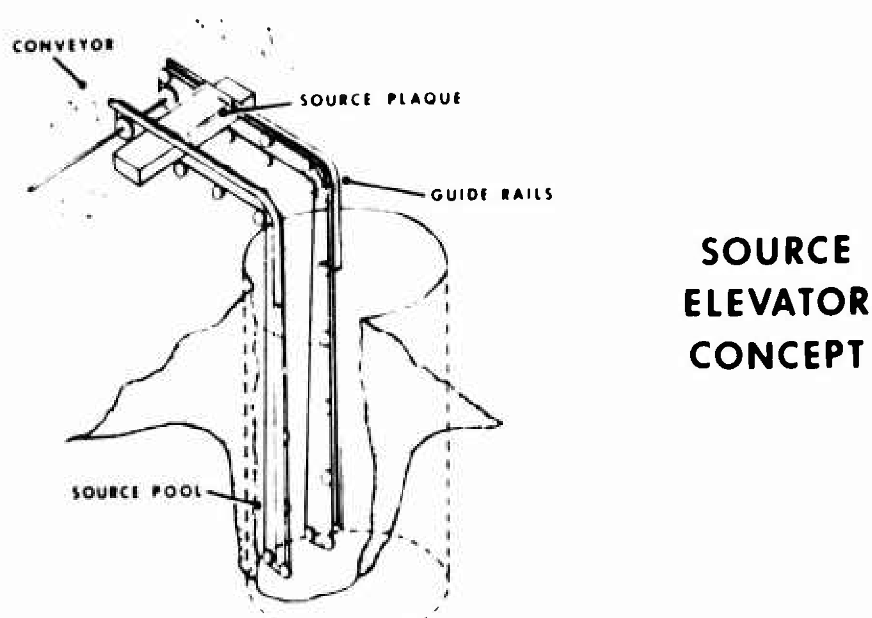

Figure 3. MPDI: Concept of source elevator.

Packaging

The Continental Can Company recently completed a survey of packaging requirements[10] for radiation pasteurized foods. Their report concludes that these requirements can be met by current technology and materials. The study, which involved the package engineering requirements of five fruit and five marine products, is based on interviews with specialists and a literature review. Container materials, container design, and specific packaging methods are reviewed; areas of future research are recommended; and future required research, subsequent to FDA approval, is outlined. As a result of this survey, a contract was let with Hazleton Laboratories to determine the effects of radiation on the selected packaging materials in order to evaluate their utility and safety. A protocol was established with FDA, a methodology was developed, and ultimately petitions will be prepared and submitted to FDA for approval of the selected packaging materials for their intended use.

The following were the selected typical commercial packaging materials:

Cellulose Acetate
Polyethylene
Polystyrene
Nitrocellulose-coated cellophane
Glassine (fruit)
Saran-coated cellophane
Paraffin microcrystalline wax
Saran
Pliofilm (rubber hydrochloride)

Extraction cells containing the packaging film in contact with the food-simulating solvents (water, alcohol, and acetic acid) were treated to a one Mrad dose of irradiation. After radiation, the cells were placed in storage under controlled condition of humidity and temperature. After removal from storage the solvent was decanted and evaporated; the residue weighed and reported as total extractives. The residues from the aqueous solvents were extracted with chloroform and, after evaporation, the weighed residues were reported as chloroform-soluble extractives. These residues were redissolved in chloroform and analyzed by infrared spectrophotometry. The results, expressed in milligrams per square inch of film, are compared to non-irradiated controls. Results varied from minus differences (i.e., the irradiated materials showed lower total extractives) to an increase of 0.50 milligram per square inch for water extracted cellulose acetate. The results were presented to an expert industry committee. The conclusion was that a radiation dose of 1 Mrad or less has no significant effect on the materials studied. We will shortly prepare a petition requesting a regulation relative to the safe use of these materials.

Wholesomeness

The U. S. Army early established an extensive research program to determine the wholesomeness of radiation-processed food. The two-year feeding tests are supported by in vivo and in vitro tests, which include growth, reproduction, hematology, histopathology, toxicity, carcinogenicity, and induced radioactivity studies. Twenty-one different foods, irradiated at sterilizing levels of 2.79 or 5.58 Mrad and stored for at least three months at room temperature, are incorporated into a basal diet as 37 per cent of the solids. Statistical analysis of the final histopathological examinations will be completed shortly. The AEC is fortunate in being able to use the Army results, where applicable, and to extrapolate to the low-dose program.

The Division of Biology and Medicine, AEC, has a program to determine the wholesomeness of other irradiated foods of interest. Existing contracts in this program are listed in Appendix 2. In a literature analysis[11] by MIT to define the wholesomeness and safety of marine products, areas of required research are identified and a protocol leading to official clearance is established. Data derived from U. S. Army studies at 3 and 6 Mrad may be extrapolated to the same class of foods for pasteurizing doses. However, further work on protein availability is required in such cases. This document, plus other recent information, is used to establish the research and development program needed to evaluate the nutritional quality and wholesomeness of the diet.

Two-year feeding studies are in operation for soft-shell clams and are planned for strawberries. Protein availability studies on haddock, crab, flounder, and clams should also lead to clearance of these food items for use.

An expanded program has been initiated at MIT and Oregon State in microbiology to determine the incidence of the various putrefactive anaerobes, including C. botulinum Type E, and the effects of low-dose irradiation on their growth and toxin production. In experiments at MIT Clostridia counts were reduced significantly by low doses of irradiation and remained below 100 per gram at both 0° and $6^{\circ}C$, even after three months in storage. This is a rather encouraging result from the public health standpoint.

The workers at Oregon State are particularly interested in the irradiation resistance of Salmonella, in the food poisoning microorganisms and their radioresistance, and in growth under the shifting ecology subsequent to irradiation.

Acceptability

Subsequent to laboratory taste testing, the acceptability of the product after being processed and handled should be determined on a large scale. Preliminary results of acceptability tests show marine products to be highly acceptable under the conditions studied. At Ft. Lee, Virginia, large-scale Army troop feeding tests are planned. These tests will involve more than

400 individuals. Comparison will be made of irradiated haddock 15 and 30 days old with its fresh counterpart. This is the first of a series of large-scale tests currently including crab, shrimp, clams, and oranges.

Petitions

Three petitions have been approved for irradiated foods (Appendix 3): one for bacon irradiated at 4.5 to 5.6 Mrad sterilizing dose with Co^{60}; one for the insect disinfestation of wheat and wheat products at 20,000 to 50,000 rads; and an amendment to the bacon petition to provide for electron beam radiation at 5 mev or less.

A petition has been filed by the U. S. Army for the use of gamma radiation to inhibit sprout development in white potatoes. Another requests a regulation to provide for safe use of electron-beam irradiation with a maximum energy of 10 mev for radiation sterilization of canned bacon. The AEC has filed a petition for the radiation treatment of bacon with sealed gamma sources having an energy of less than 2.2 mev (e.g., Cesium-137). Through Hazleton Laboratories, the AEC and the Army jointly requested the FDA to allow irradiation of citrus fruit, to control spoilage, with sealed gamma sources up to 2.2 mev.

Future Plans

The results obtained in this program show the continued promise of this developing technology. Based on these results, we are confident of the future commercialization of the process.

The clearance of radiation sterilized bacon on 8 February 1963, signaled an increased participation by industry; first by equipment manufacturers and second, by food processors. However, commercialization of the process has not yet occurred. There still remains much to be done by way of process development for some of the various food items showing promise. Furthermore, we will continue to press toward clearance for human consumption of more food groups so as to provide a broader basis for application.

APPENDIX 1

Contracts in Effect in the AEC, Division of Isotopes Development, Program on Low-dose Radiation Pasteurization of Food

		Subject of Study	Contractor
I.	Fruit	Studies on Gamma Radiation Treatments for the Control of Post-Harvest Diseases of Fresh Fruits and Vegetables	USDA, Agricultural Marketing Service, Chicago
		Radiation Technology in Conjunction with Post-Harvest Procedures as a Means of Extending the Shelf Life of Fruits and Vegetables	University of California, Davis, California
		Radiation Pasteurization of Foods	University of Michigan (also Michigan State University and Bureau of Commercial Fisheries, Ann Arbor, Michigan)
		Effect of Low Level Irradiation Upon the Preservation of Food Products	University of Florida, Agricultural Experiment Station, Gainesville, Florida
		Irradiation of Fruits and Vegetables with AECL Mobile Irradiator	USDA, Fresno, California
II.	Fish	Radiation Preservation of Fishery Projects	USDI, Bureau of Commercial Fisheries, Gloucester, Massachusetts
		Application of Radiation Pasteurization Processes to Pacific Crab and Flounder	USDI, Technological Laboratory, Seattle, Washington

		Subject of Study	Contractor
II.	Fish (cont'd.)	Extension of Storage Life and Growth of Type E. Clostridium botulinum in Radiation Pasteurized Marine Products	MIT, Department of Nutrition, Food Science and Technology, Cambridge, Massachusetts
		Radiation Pasteurization of Shrimp	Louisiana State University, Baton Rouge, Louisiana
		Effects of Irradiation on the Microbial Flora Surviving Irradiation Pasteurization of Shrimp	Oregon State University, Department of Fooa Science and Technology, Corvallis, Oregon
		Radiation Pasteurization of Foods	University of Michigan (also Michigan State University and Bureau of Commercial Fisheries, Ann Arbor, Michigan)
III.	Market Evaluation	Marketing Feasibility Study of Radiation Pasteurized Fresh Strawberries, Peaches, Tomatoes, Grapes, Oranges, and Grapefruit	USDA, Economic Research Service
		Study of Attitudes and Reactions of Potential Consumers of Irradiated Fishery Products	USDI, Bureau of Commercial Fisheries
		Current Status and Potential of Irradiation to Prevent Potato Sprouting	Western Nuclear Corp., Idaho Falls, Idaho
IV.	Petition	II. Preparation and Submission of Petition of Safe Use of Radiation on Citrus Fruits and Marine Products	Hazleton Laboratories, Falls Church, Virginia
V.	Packaging	I. Extraction Study of Packaging Materials and with Irradiated Foods	Hazelton Laboratories, Falls Church, Virginia
VI.	Design and Construction	Brookhaven National Laboratory	

APPENDIX 3

U. S. Food and Drug Administration Regulations and Related Actions

1. Gamma Radiation for the Processing of Food, Food Additive Petition (FAP) 890—filed by Department of the Army, Quartermaster Research and Engineering Center, Natick, Massachusetts. On February 15, 1963, a regulation was issued in the Federal Register that radiation by cobalt-60 may be used to sterilize bacon in coated cans by irradiation to an absorbed dose of 4.5 to 5.6 Mrads. 33 FR 1465

2. Gamma Radiation for Treatment of Wheat—filed by Lloyd E. Brownell et al. A regulation was issued in the Federal Register, August 21, 1963, including the following: gamma radiation from sources with maximum energy not to exceed 2.2 million electron volts, to provide an absorbed dose from 20,000 to 50,000 rads, may be safely used for the irradiation of wheat and wheat products for control of insect infestation. 28 FR 9208

3. Electron Beam Radiation for Processing of Food. Submitted by the General Electric Company, Milwaukee, Wisconsin. On August 30, 1963, a regulation was issued in the Federal Register to permit the irradiation of canned bacon using an electron accelerator producing a beam of electrons at energy levels not exceeding 5.0 mev. 28 FR 9526

4. Notice of Filing of Petition Regarding Food Additive Gamma Radiation. FAP 1132 Federal Register, June 6, 1963. Petition filed by Department of the Army, QMR&E Command, Natick, Massachusetts, proposing a regulation for use of gamma radiation at energy levels of not more than 2.2 mev for inhibiting sprout development in white potatoes with an absorbed dose of 5,000-10,000 rads. 28 FR 5588

5. Notice of Filing of Petition Regarding Food Additive FAP 1205, Federal Register, August 23, 1963. Petition filed by the Department of the Army, QMR&E Command, Natick, Massachusetts, proposing a regulation to provide for the safe use of electron beam radiation with a maximum energy of 10 mev and a dose of 4.5 Mrad for the radiation preservation of canned bacon. 28 FR 9329

6. Letter: Reply USFDA to USAEC letter dated August 26, 1963, requesting an amendment to the regulation permitting "Gamma Radiation for the Processing of Food" as published in 33 FR 1465 to include "Processing by sealed sources of gamma radiation having an energy level of less than 2.2 mev;" reply designates USAEC letter request as FAP No. 1226.

7. FAP 1233 filed September 13, 1963, by Hazleton Laboratories jointly on behalf of the AEC and Department of Army; requested gamma radiation from sealed sources with maximum energy not to exceed 2.2 mev, to provide and absorbed dose of about 100,000 rads for lemons and 200,000 rads for oranges to be safely used for the irradiati n control of spoilage.

8. FAP 1297 filed November 29, 1963 by Hazleton Laboratories on behalf of USAEC. Covering the general classes of packaging materials for potential use with irradiated foods which were evaluated.

9. FAP 1276 filed by High Voltage Engineering Corporation. Provides for the safe use of electrons at energy levels not to exceed 5 million electron volts and with an absorbed dose of 20,000 to 50,000 rads for control of insect infestation in wheat and wheat products.

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7. Radiation Preservation of Selected Fruits and Vegetables, Stanford Research Institute, USAEC Report No. SRIA-30 (1961).

8. Study Report Cobalt-60 Bulk Grain Irradiator, Brookhaven National Laboratory, BNL 810 (T-312).

9. Study Report Shipboard Cobalt-60 Radiopasteurizer for Marine Products, Brookhaven National Laboratory, BNL 808 (T-311) (1963).

10. Continental Can Company, Survey of Packaging Requirements for Radiation Pasteurized Foods, TID-15144, Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C.

11. A Literature Survey of the Effects of Ionizing Radiation on Sea Foods with Respect to Wholesomeness Aspects, Massachusetts Institute of Technology, USAEC Report No. TID-11610 (1961).

WHOLESOMENESS, NUTRITIONAL ADEQUACY, AND MICROBIOLOGY

Captain Leo A. Whitehair

Introduction

The Atomic Energy Commission's Division of Biology and Medicine supports an important portion of the AEC Radiation Pasteurization of Foods Program which is closely oriented with the Division of Isotopes Development activities described today by Dr. Shea, Major Dietz, and Mr. Machurek. The contract studies in the Biology and Medicine program pertain to the areas of wholesomeness, microbiology, biochemistry and physiology of low-dose irradiated marine products and fruits.

The activities in this program can best be described by briefly summarizing each of the contracts currently in effect in these areas.

Animal Feeding (Wholesomeness) Studies

Earlier this year, a Protocol for Animal Feeding Investigations of Radiation-Pasteurized Foods in the Commission's Program was prepared within the Division of Biology and Medicine. This protocol for biological evaluation of protein utilization and long term chronic toxicity studies was prepared in accordance with requirements outlined by the Food and Drug Administration. Two contracts for studies of this nature are now in effect.

The first of these two contract studies is at the University of Illinois (Investigator: Dr. E. L. Reber). Biological evaluations of protein utilization are being conducted on radiation pasteurized haddock, flounder, crab, and soft shell clams. These evaluations are of a short term nature and will essentially complete the wholesomeness data requirements for haddock, flounder, and crab since the Army program has already completed two-year wholesomeness studies on related marine species (cod, tuna, and shrimp). A two-year feeding study on irradiated soft shell clams (representative of the mollusks) will be conducted. Three species of animals (rat, dog, chicken) will be utilized in this study.

A contract for two-year feeding studies on radiation pasteurized strawberries is now in effect with Industrial Bio-Test Laboratories (Investigator: Dr. Margaret Ives). The protocol described earlier will again be adhered to in these studies.

Arrangements for radiation services to be performed at the Natick facility have been made through Dr. Josephson and Mr. Schaller for foods used in the Commission's wholesomeness feeding studies. This arrangement is planned to continue until the AEC radiation facility at Gloucester, Massachusetts, is in operation. We sincerely appreciate the use of the fine facility here at Natick.

University of Michigan
(Investigators: Drs. John Graikoski and Lloyd L. Kempe)

Past work on this contract has shown that Clostridium botulinum Type E spores are more easily killed by gamma radiation than Types A and B under similar conditions. The D value for Type E spores in phosphate buffer was approximately of the order of 134,000 rads. The heat resistance of Type E spores was found to be much less than that of Types A or I .

Comparisons made of toxin production from six different strains of Cl. botulinum Type E in various media showed that both the strain of organism and the type of media affect the amount of toxin produced. The optimal temperature for toxin production was found to be about 20°C, with very little toxin being produced at 37°C.

Radiation resistance of the several strains of Type E spores has been compared in phosphate buffer and sea food substrates. Studies were initiated during the past year on effects of media variation, carbohydrate composition, and pH of culture media on toxin synthesis.

Low temperature incubation experiments on Type E spores which will be conducted by Drs. Graikoski and Kempe during the coming year are being extended to include additional substrates, such as irradiated smoked fish and fresh-water fish as well as marine fish. Spores of six Type E strains and Types A and B will be included in these studies. The heat resistance of three strains of Type E is being compared to learn whether heat resistance correlates with radiation resistance. Studies on effects of media composition and media variation on spore germination are being continued.

Continental Can Company, Inc.
(Investigators: Drs. Bruce Morgan, Wayne Segner, Clarence F. Schmidt)

The purpose of this study is to evaluate public health hazards involved in the prolongation of refrigerated storage life of food by application of low doses of ionizing radiation. Factors which govern and control public health hazards of Clostridium botulinum Type E in refrigerated foods are to be established.

Results of the first year's activities on this study can be summarized as follows:

1. Spore crops of four strains of Cl. botulinum Type E have been prepared with viable spore counts ranging from 22 to 35 billion spores.

2. Varying the substrate has resulted in marked differences in the total growth spore crop yield and outgrowth time of Clostridium botulinum Type E.

3. The effects of salt concentration, temperature, and inoculum level upon growth inhibition have been delineated.

4. Preliminary results on the pH inhibitory effect upon Cl. botulinum Type E have been secured.

5. Preliminary results on the thermal resistance in the range of 166°-$186^{\circ}F$ for spores of Cl. botulinum Type E have been obtained.

Studies which are now in progress and which are planned during the current year are as follows:

A. Primary Factors

1. Continue exploration of factors allowing for spore germination and outgrowth between 34° and $50^{\circ}F$.

2. Continue the study of relationship between time of appearance of outgrowth, time of first detectable toxin formation, and temperature of storage. Homogenized marine products will be included as test substrates.

3. Conclude work on pH relationship of the substrate to growth and toxin formation between 34° and $50^{\circ}F$.

4. Define the optimum conditions for detection of presence of Type E toxin in food products.

5. Continue delineation of factors affecting the sporulation of Cl. botulinum Type E in terms of production of spore crops and their stabilization.

B. Secondary Factors

1. Initiate determination of thermal and radiation stability of Type E toxin (free toxin and prototoxin).

2. Further define the thermal resistance of type E spores in the temperature range required for enzyme inactivation by heat (150°-$180^{\circ}F$).

3. Correlate the effect of growth and sporulation conditions on radiation resistance of Type E spores.

4. Determine the effect upon Type E growth and toxin production and stability of this toxin when in a mixed flora of other organisms capable of growth at 50°F and below.

C. Related Factors

1. Define the radiation resistance of Type E spores which survive various levels of heat shock.

2. Determine the low temperature growth potential of spores of Types B, C, and D.

3. Determine the natural distribution of Type E spores in soil and other selected media.

University of Washington
(Investigators: Drs. A. M. Dollar and J. Liston)

The primary objectives of this study are in two areas: microbiology and biochemistry. These two areas are closely related and the research efforts are intended to resolve mutual questions. During the first year, efforts were concentrated on the bacteriological safety of radiation-pasteurized sea foods and on basic microbiological processes involved in the irradiation procedures. In particular, the sensitivity of bacteria of public health significance to low-dose irradiation in relation to the suspending medium and the qualitative effects of pasteurizing treatments on the natural bacterial flora have been investigated. In the safety evaluation studies, samples of fish and shellfish were tested before and after radiation and during subsequent storage at 33° and 42°F. Tests were for total count, coliform and E. coli count, Salmonellae and Coagulase staphylococci count, Enterococcus count, total anaerobic and Clostridium perfrigens counts.

The main microbiological objective is to study both spoilage and potentially pathogenic organisms and determine (1) their sensitivity to radiation, (2) outgrowth after radiation, and (3) to obtain estimates of physiological changes.

The biochemical studies serve to complement the microbiological studies. Principal objectives of the biochemical studies now in progress are to identify products and mechanisms involved in the tissue changes and relate these to radiation dose levels. The effect of radiation dose on mechanisms and products responsible for flavor changes, particularly the changes in the nucleotides will be studied. Secondly, since water is intimately associated with microbiological and tissue changes (including oxidative changes), the effect of free and bound water content on the changes in protein and other components will be studied. Quantitative measurements of biochemical changes in relation to radiation dose levels and dose rates will be made.

The over-all objective of these studies is to determine parameters of radiation dose rates and dose levels that will achieve maximum kill of bacteria with minimum damage to the essential properties which will yield a fresh-tasting, wholesome fish.

University of California (Davis)

Two general areas of research are currently being conducted:

1. Radiation Biochemistry of Fruits

These are basic studies to explore the effects of ionizing radiation on the cellular and intracellular structure and metabolism of fruit tissues. Studies currently in progress include (1) a survey of intracellular protein nitrogen distribution as affected by radiation, (2) isolation of active mitochondrial systems from irradiated tissues, (3) extraction and identification of structural fatty acids from mitochondria of irradiated and control tissues, (4) changes in soluble enzymes, particularly transaminases, as induced by radiation, and (5) a study of pectin changes with the thought of correlating pectin, auxin, and sulfhydryl relationships to effects of radiation on cellular membranes, softening of tissues, and plasticity. Research in this area is under the direction of Dr. Roger J. Romani.

2. Radiation Physiology and Microbiology of Fruits

These are investigations dealing with specific problems arising from the radiation pasteurization of fruit. Studies include (1) determinations of ethylene production as induced by radiation, (2) effects of radiation on fruit pathogens, their resistance, recovery and general response to irradiation, (3) measurements of fruit respiration during irradiation, (4) immediate effects on sulfhydryl and ascorbic acid levels and changes in pectin states as induced by irradiation. Research in this area is under the direction of Drs. Neil Sommer, Edward C. Maxie, and Roger J. Romani.

Cornell University (Geneva Agricultural Experiment Station)
(Investigator: Dr. Leon Massey)

The purpose of this study, which has just recently been initiated, is to define and explain the changes which occur in fresh fruits and vegetable tissue associated with the extension of shelf life of such items by means of ionizing radiation.

The investigation will include (1) the definition of such changes in terms of chemical, biochemical, and physiological responses to radiation, (2) alleviation of the undesirable changes by modification of environmental conditions (e.g., temperature, ambient atmospheric composition, etc.), and (3) selection of resistant varieties of fruits and vegetables and states of maturity. Emphasis

will be directed but not restricted to exploring certain promising leads such as mold prevention in fresh strawberries and grapes and storage scald and brown core prevention in apples.

Experiments will be conducted on softening changes which occur in plant tissue during and after exposure to radiation with special emphasis on elimination of this defect. Research will also be performed on alteration of respiratory activity, production of volatiles, and other processes which are known to relate directly to shelf life. Attempts will be made to relate these changes to alterations in biochemical phenomena, such as enzyme activity and substrate availability.

Preliminary results indicate that post-harvest shelf life of apples may be prolonged by pasteurizing doses of gamma radiation through control of storage scald and brown core. In addition, it appears that apples, when irradiated, exhibit changes in volatile production rate which may also be of indirect benefit. These observations will be further explored to define the responses in terms of both the nature of the changes observed and the basic cause of these changes.

Bureau of Commercial Fisheries, Gloucester Laboratory
(Investigators: Drs. Joseph Slavin, Louis Rcnsivelli, and Maynard Steinberg)

Earlier work (supported under contract by the Division of Biology and Medicine and conducted at the Gloucester Laboratory by Drs. Slavin and Steinberg) on the stability of amino acids and B-vitamins in both irradiated clams and haddock showed little or no changes in these nutrients because of the use of radiation.

The present work at Gloucester within our program concerns gas chromatographic analyses of volatile compounds from irradiated and control fish. The tests are designed to establish chromatogram patterns which, it is hoped, can be correlated with detailed results of organoleptic testing on the same fish products. The investigators have made gas chromatograms of known, pure, pertinent chemical compounds to use as standards for the identification of unknown volatile components in fish.

University of California (Berkeley)
(Investigator: Dr. W. Duane Brown)

The objective of this study is to define the changes in the protein moiety of hemoproteins as a result of ionizing radiation. Highly purified solutions of myoglobins from yellowfin tuna and sperm whale have been studied during the first year.

Myoglobins are well suited for studies attempting to elucidate structural changes in protein since the amino acid composition, sequence, and tertiary

structure is known for at least one. Myoglobins are responsible (together with various derivatives) for the color of most fish and meat products. They have an active physiological function, are readily prepared in highly purified form, and have been the subject of a number of comparative studies. Tuna myoglobins contain one cysteine residue per molecule, while mammalian myoglobins do not; therefore, the use of both allows model systems for the study of the effects of ionizing radiation on SH (sulfhydryl) groups in intact proteins.

The second year of this study has just begun. Efforts will be made to identify peptide fragments that are split, apparently specifically, from purified myoglobins in solution. It will be determined if this cleavage, and other alterations affecting electrophoretic, chromatographic, and ultracentrifuge sedimentation properties, affect heme derivative formation and oxygen-binding capacity. These studies will be extended to irradiation of myoglobin *in situ* in muscle, followed by its extraction, purification, and determination of any changes in biophysical properties.

Conclusion

The contract studies which have been described are designed to provide vital data concerning the wholesomeness and biological safety, microbiology, and biochemistry of low-dose irradiated foods. The findings of these studies will be welded with previous results obtained in the Army's program and results of the studies currently being supported by the Division of Isotopes Development. We are confident that results of these combined efforts will lead to the successful use of radiation as a means of extending the storage life of perishable foods.

DEVELOPMENT OF RADIATION FACILITIES

Major George R. Dietz

Introduction

The primary emphasis thus far in food irradiation research has been the determination of radiation technology and the establishment of wholesomeness and safety. Like any other research and development program aimed at an end process, there will follow an evolution from the laboratory process to full commercialization. Two of the notable steps in this unfolding will be the establishment of proto-commercial radiation facilities to extend laboratory results to commercial-like processing conditions, and finally to establish facilities capable of utilization in actual commercial operation. Furthermore, these latter facilities must be designed, constructed, and operated in such a manner so as to provide an end product which is not only acceptable, but can be expected to be economically competitive with other preservation methods. If other competitive methods are not applicable, then the end product must be unique either in storage quality or in inherent characteristics which reduce spoilage losses. In either of these cases, costs must still be acceptable to the industry and to the consumer.

A major portion of the AEC program has been devoted to the development of a family of radiation facilities. Initial emphasis was on design and construction of research irradiators capable of supporting food irradiation studies. Three such units, to be discussed more in detail, have already been installed at research sites, and another is currently being installed at a fourth site.

Current emphasis is on proto-commercial facilities either under design or construction, or planned for construction as the program develops. This type of facility is intended to translate laboratory data to semi-production or pilot plant operation to prove out laboratory data on a near commercial scale and to aid in the determination of the economics involved. Included in this category are mobile or transportable units, a Marine Products Development Irradiator (MPDI), and a grain irradiator. AEC is also actively exploring prospects of establishing a moderate semi-production plant in the state of Hawaii. Irradiation as a solution to quarantine control has shown very encouraging initial results. Extension of quality and shelf life of products such as pineapples, mangoes, and papayas appear to be favorable but require further investigation. If continued research bears out these favorable results, and industry interest remains high, the program will most likely be

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extended to include a pilot plant operation. A final category deals with a type or types of irradiators which may be classed as highly specialized, or applicable in unique situations. An example is an on-board ship irradiator.

With this introduction to the over-all concept of irradiator development, I will discuss each a bit more in detail.

Research Irradiator (Fig. 1)

This unit is a highly versatile, safe, easily operated food research irradiator which is installed at or immediately adjacent to the research site. Its proximity to the research site and ready availability to those conducting research are especially advantageous in that radiation parameters can be determined on foods in their natural and fresh state, without the need of artificial preservation during transit to some remote irradiation site.

The research irradiator is essentially a pool type gamma source of approximately 30,000 curies of cobalt-60 and has a capacity of irradiating approximately 75 lb of food at a dose of one million rads per hour. One irradiation chamber is provided with both temperature and atmosphere control to facilitate experimental work. A distinct advantage of the irradiator is simplicity of operation. A central control panel is so constructed that a moderately trained individual can operate the facility without possibility of endangering himself or others from radiation. The irradiator is of moderate cost and is readily installed at any particular site.

It is designed to utilize cobalt-60 sources in the range of 25,000 to 50,000 curies in the form of two rectangular plaques. The

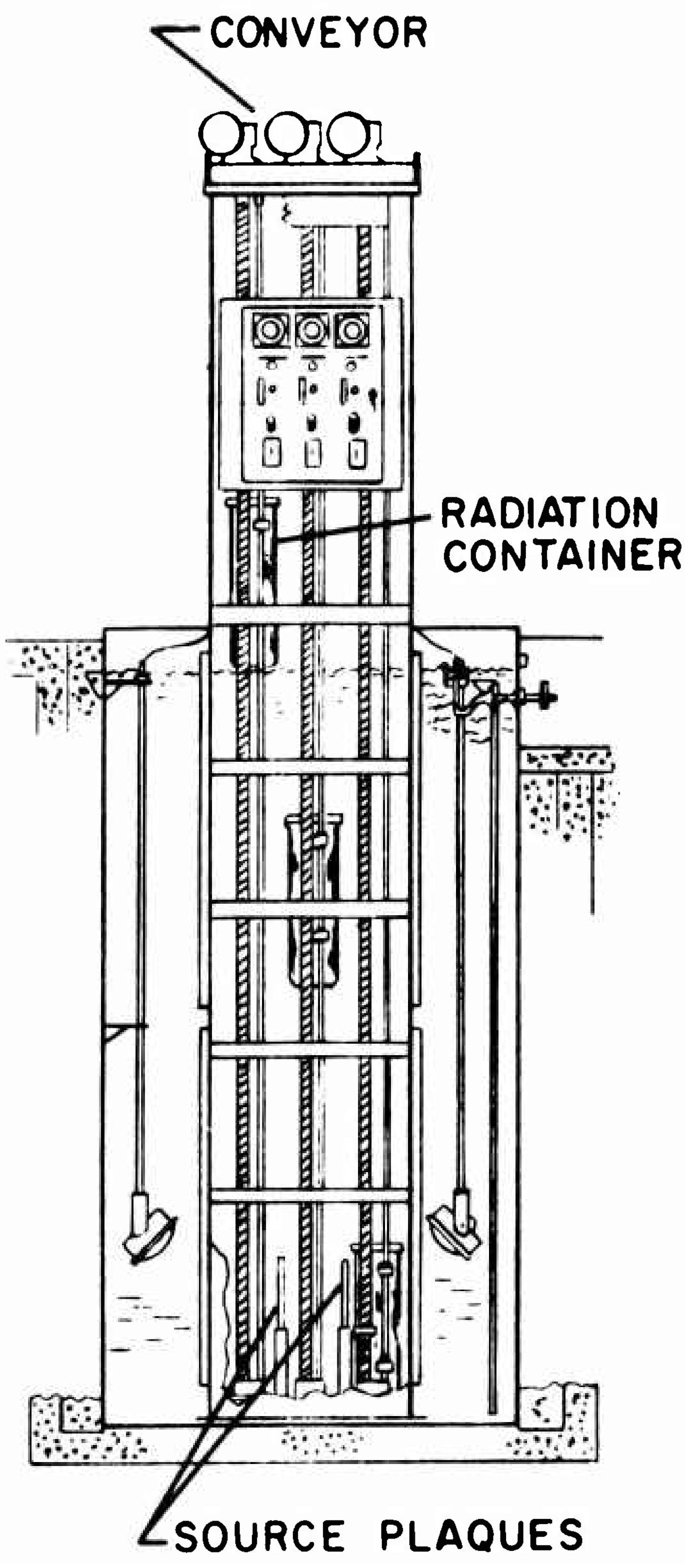


Figure 1. Brookhaven National Laboratory University Research Irradiator.

sources are installed in racks at the bottom of a 12-foot deep stainless steel tank filled with water. Watertight boxes capable of accommodating packages measuring up to 14" x 18" x 5" are lowered into position between and outside of the two plaques. The period of irradiation can be pre-set, and the irradiation performed automatically. Safety instrumentation and a water purification unit are included in this facility.

Four of these units have been installed and support research as follows:

Location	Researchers
Massachusetts Institute of Technology	MIT and USDI, Gloucester, Massachusetts
University of California (Davis)	University of California
University of Washington	University of Washington and USDI, Seattle
University of Florida	University of Florida (under installation)

Table 1 summarizes the characteristics of the research irradiator.

TABLE 1

Research Irradiator[a] Characteristics

Purpose: Immediate availability for research support.
Type: Double plaque, pool storage.
Source: 3,000-40,000 curies of cobalt-60.
Capacity: 75 lb/hr at 1 Mrad.
Cost: $35,000 plus source.

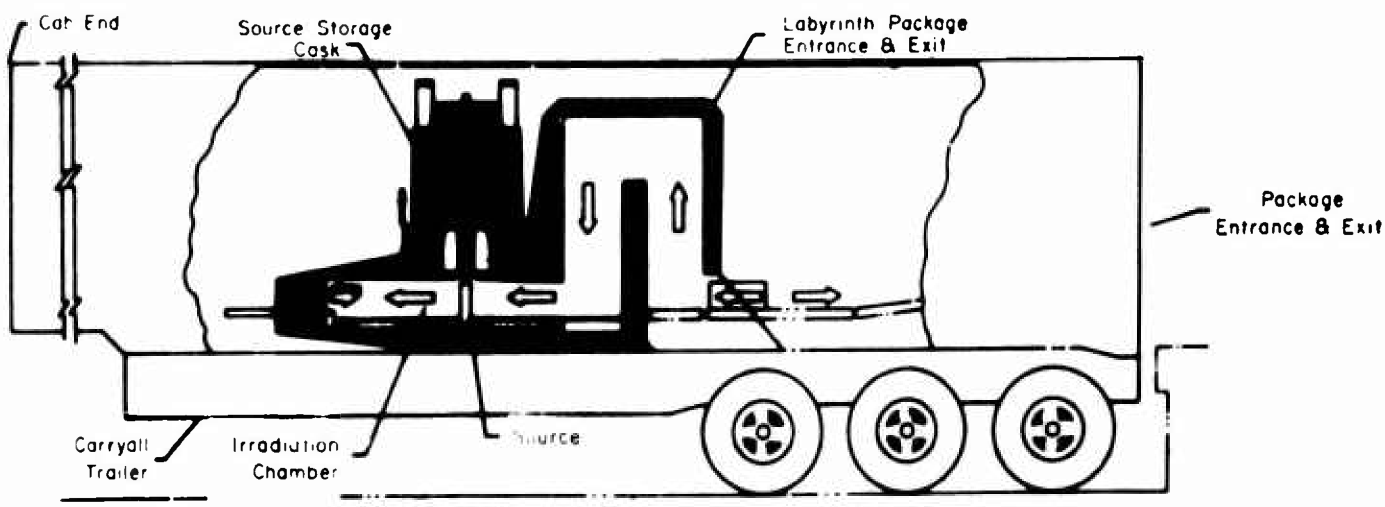
[a]Manufacturer: Processing Equipment Corporation, Lodi, New Jersey.

Transportable Units

Two truck mounted transportable or mobile units have been studied for use with the food program. Conceptual design studies have been completed for a unit utilizing cobalt-60 and one utilizing an electron source. The purpose of these units will be to demonstrate on a wide scale the applicability of food irradiation, especially to the fruit industry. The transportable unit will have the capability of following crop harvests as they progress throughout a particular region. It is expected also that a clearer determination of economics will evolve from their availability and use.

The AEC food program budget for this fiscal year (FY 1964) contains funds for the design and construction of the mobile cobalt unit. The unit under consideration (Fig. 2) will be a multiple pass system, but the ultimate design will be determined as detailed engineering drawings are completed. Several concepts have been presented to AEC for consideration. In general, this type of irradiator, self-contained, would utilize up to 150,000 curies of cobalt-60, weigh in the neighborhood of 50 to 70 tons, and have a production capacity of up to 2,000 lb per hour of fruits irradiated to a dose of 250,000 rads.

PROCESSING CAPACITY - 5000 LBS / HRS
RADIATION SOURCE - 100,000 CURIES OF COBALT 60
WEIGHT OF SHIELDING A CASK
WEIGHT OF TRACTOR & TRAILER - 36,000 LBS
AUXILIARY EQUIPMENT - 10,000 LBS
TOTAL WEIGHT - 93,700 LBS



TRANSPORTABLE IRRADIATOR. FUELED WITH APPROXIMATELY 1,000 CURIES OF COBALT-60, THIS CONCEPTUAL DESIGN DEPICTS THE CURRENT CONCEPT OF A TRANSPORTABLE IRRADIATION UNIT, DESIGNED TO SUPPORT THE COMMISSION'S RADIATION PRESERVATION-OF-FOODS PROGRAM. WITH A SEMI-PRODUCTION CAPACITY, THE UNIT WILL BE CAPABLE OF DEMONSTRATING THE PRACTICAL FEASIBILITY OF RADIATION PASTEURIZATION AS WELL AS AIDING IN THE DETERMINATION OF THE ECONOMICS INVOLVED.

Figure 2. Transportable Irradiator. Fueled with approximately 1,000 curies of cobalt-60, this conceptual design depicts the current concept of a transportable irradiation unit, designed to support the Commission's radiation preservation-of-foods program. With a semi-production capacity, the unit will be capable of demonstrating the practical feasibility of radiation pasteurization as well as aiding in the determination of the economics involved.

Current AEC plans are to proceed with design and construction of this mobile irradiator as quickly as funds become available. Final completion is expected in time for the 1965 spring fruit harvest in the California area.

Table 2 summarizes general mobile irradiator characteristics.

Grain Irradiator (Fig. 3)

Disinfestation of grains is an easily accomplished task with the use of irradiation, at least from a technological standpoint. Clearance of radiation processed wheat and wheat products by FDA makes wheat irradiation a potential candidate for immediate commercialization. The greatest obstacle to commercialization is the economics of wheat irradiation. At the moment, it appears to

TABLE 2

Mobile Irradiator[a] Characteristics

Purpose:	Wide scale demonstration of feasibility of radiation processing of fruits, economic determinations.
Type:	Truck mounted, mobile.
Source:	100,000 to 150,000 curies of cobalt-60.
Capacity:	One ton/hr, dose of 250,000 rads.
Cost:	$350,000 complete (estimated).

[a]Designs completed by Associated Nucleonics, Inc., Garden City, L.I., New York, under AEC contract; Vitro Engineering Corp., New York, inhouse. Construction to begin in FY 1964.

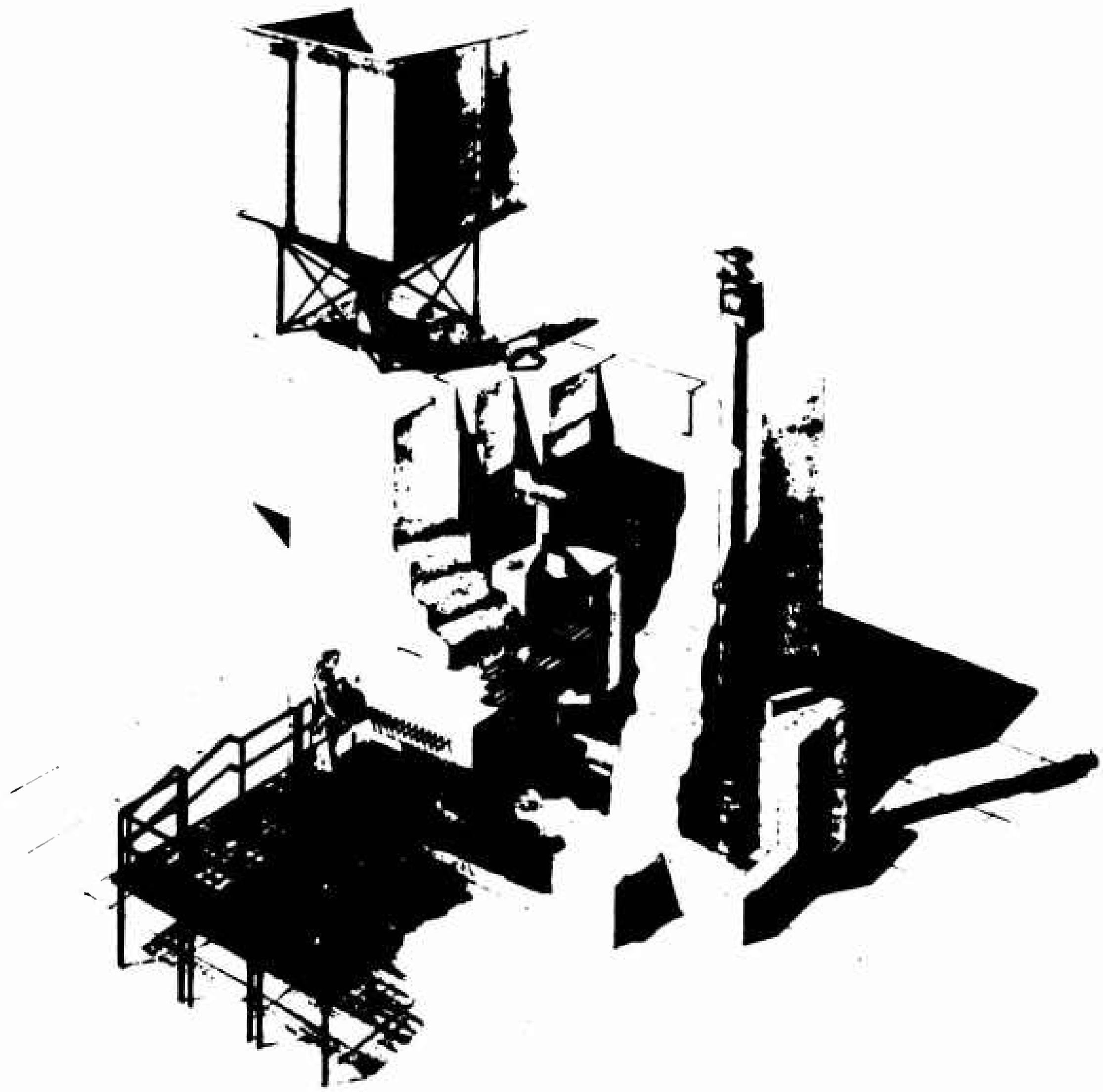


Figure 3. Cobalt-60 bulk grain irradiator.

be cheaper to absorb present grain losses than to process for insect control, although a more insect free and aesthetic product could be produced. It is primarily the low initial cost per unit of wheat which precludes additional processing costs.

An alternative to the processing of bulk wheat is the processing of milled flour or other packaged wheat products. To further determine what role radiation might play in the field of wheat and grain irradiation, the AEC in cooperation with the U. S. Department of Agriculture is establishing a program designed to help answer this question. To support this research, AEC will provide a cobalt-60 unit capable of irradiating both bulk wheat and packaged products. Initial design has been completed, and funds for detailed design and construction are expected to be forthcoming in this year's (FY 1964) budget. A tentative site selection of the USDA's Entomological Research Center in Savannah, Georgia, has been made.

TABLE 3

Grain Irradiator[a] Characteristics

Type: Continuous grid system.
Source: 17,000 curies cobalt-60.
Dose: 15,000-25,000 rads.
Capacity: 5,000 lb/hr.
Cost: $200,000 total cost (estimate).

[a]Designer: Vitro Engineering Corp., under AEC contract. Construction to begin in FY 1964.

Central or Inplant Irradiator

As a final member of this irradiator family, the Marine Products Development Irradiator (MPDI) is currently under design and construction at Gloucester, Massachusetts. This is a semi-production fixed facility with the capability of processing up to 1,000 lb per hour of product at a 500,000 rad dose. Its use is being directed primarily toward supporting that part of the program on preservation of sea foods, and capabilities will include processing large amounts of foods for shipping, storage, and other large-scale tests. Its basic purpose is to demonstrate to the fishing industry the feasibility and advantages of radiation processing and to aid in the determination of economics related to the radiation process.

The MPDI is scheduled for completion in late summer of 1964. It is designed to utilize a cobalt-60 source of approximately 250,000 curies. In general, it is a rectangular, one story facility of slightly under 4,000 square feet, divided into a general building area and an irradiation cell. A floor plan is included to indicate specific areas and features (Fig. 4).

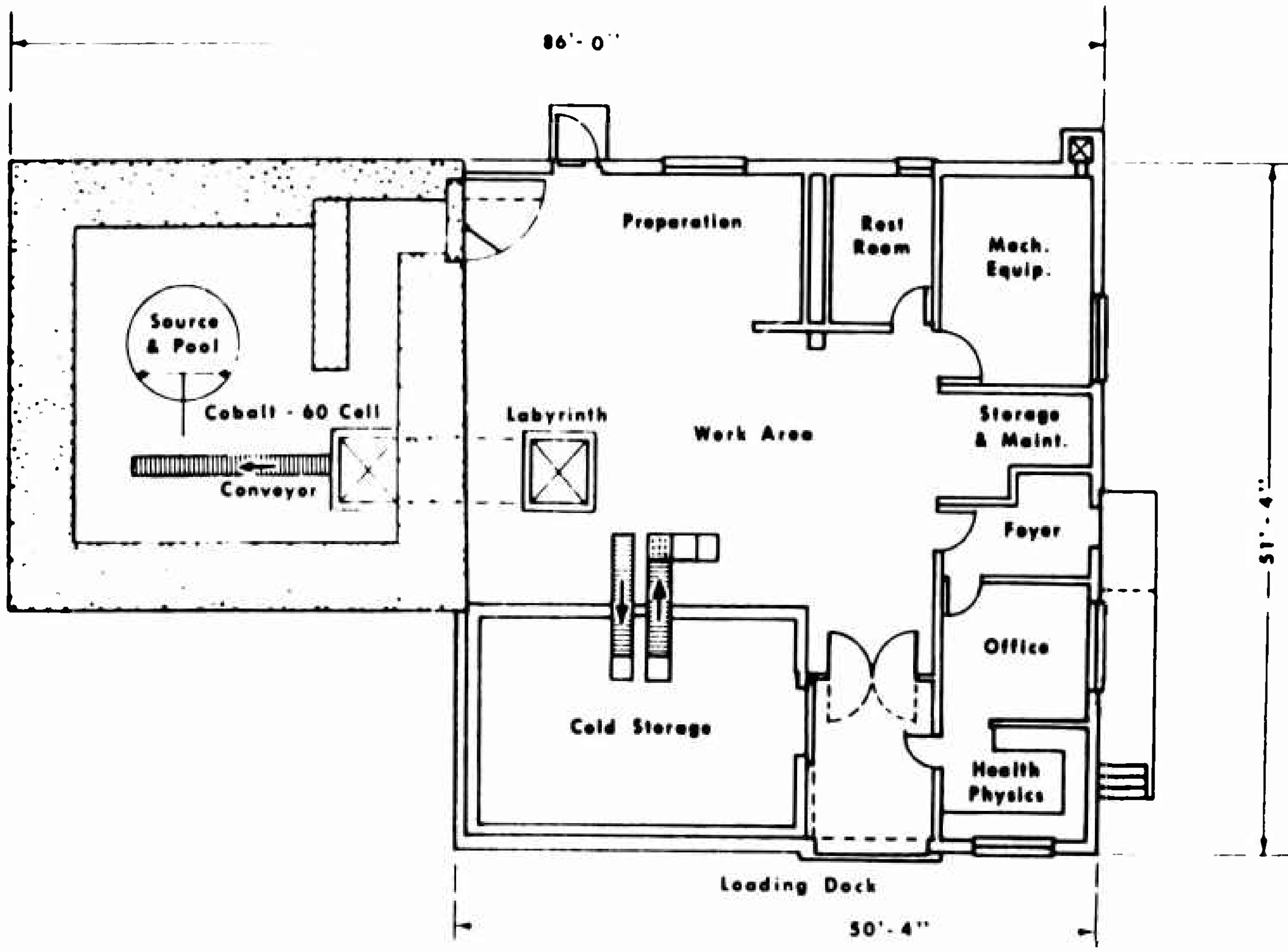


Figure 4. MPDI floor plan.

The cobalt is in a rectangular array and normally stored vertically in a water filled storage pool. In the irradiation position, the source is in a horizontal attitude, while packages being irradiated pass over and under (Fig. 5). Fillet boxes of 10, 20, and 30 lb capacity will primarily be utilized, although there is sufficient flexibility to permit irradiation in other containers such as a 6" x 24" x 30" - 100 lb package. Table 4 summarizes MPDI characteristics.

TABLE 4

MPDI[a] Characteristics

Purpose: Semi-commercial sea food irradiation.
Type: Multiple pass, pool storage.
Source: 250,000 curies cobalt-60.
Capacity: 1,000 lb/hr at dose of 500,000 rads.
Cost: $600,000 complete.

[a]Completion in late summer, 1964. Architect Engineer: Associated Nucleonics, Inc.

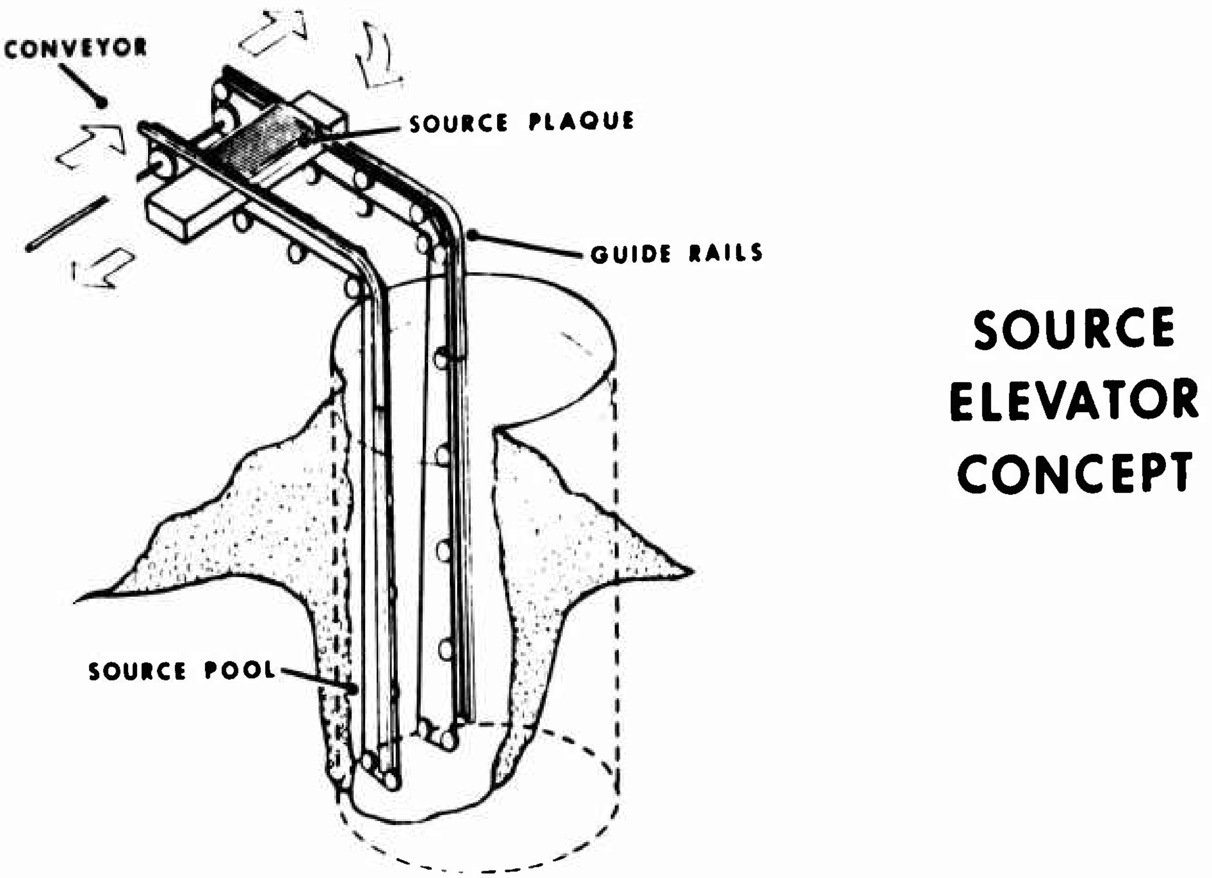


Figure 5. MPDI: Concept of source elevator.

On-Board Ship Irradiator (Fig. 6)

Earlier fisheries marketing studies have confirmed the opinion that radiation should take place as soon after catch as possible. While a land-based unit such as the MPDI represents the earliest time after landing that irradiation could take place, there is the alternative of irradiation at sea. Reduction of bacterial counts on either processed or unprocessed whole fish could be accomplished by the application of a light (100,000 rad) dose soon after catch, and the application of a second moderate dose after processing and packaging.

A "mother ship" concept, wherein one ship will provide radiation services to many other fishing trawlers, has thus far received considerable attention. Based upon advice and encouragement received from the USDI, the AEC had conducted preliminary design studies of an on-board ship irradiator which could be adapted either to a modified existing trawler, or to one of several new ships to be added to the fishing fleet within the next two to three years. Any final decision on further development of this type unit would, of course, depend heavily on the success of the MPDI and the related enthusiasm of the fishing industry.

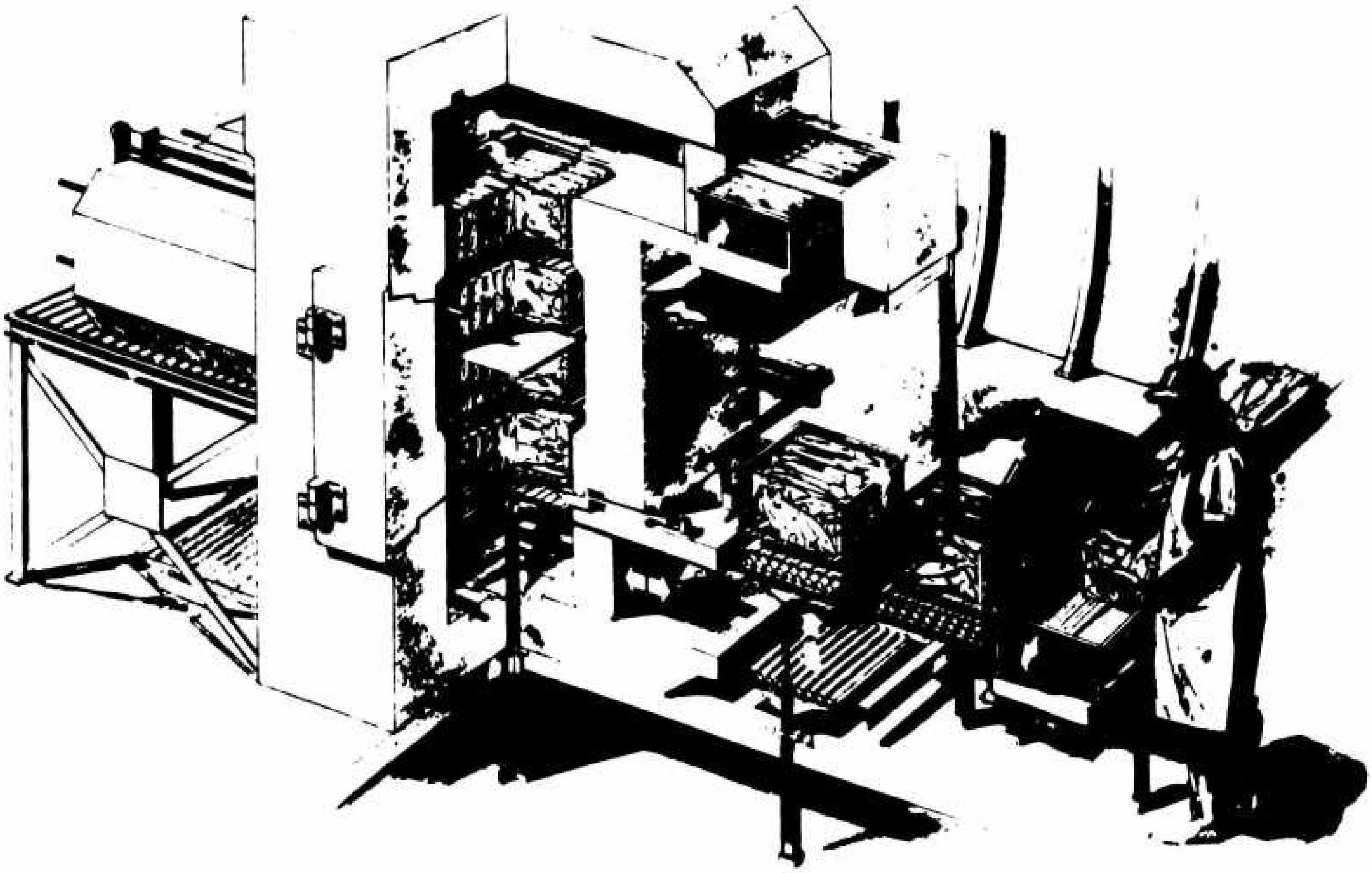


Figure 6. Cobalt-60 shipboard fish irradiator.

Table 5 summarizes the characteristics such an irradiator might include.

TABLE 5

On-Board Ship Irradiator[a] Characteristics

Type: Movable source slab, cask storage.
Source: 360,000 curies of cobalt-60.
Capacity: 7500 lb of fish/hr, dose 75,000 to 150,000 rads.
Size: 5' x 10' x 12'.
Weight: 75 tons.
Cost: $400,000 plus source (estimated).

[a]Vitro Engineering Corp. under AEC contract.

Hawaiian Irradiator

Consideration of a pilot plant in the Hawaiian area is, at present, only in the most preliminary stages of planning and is dependent on further favorable

laboratory research results. While several initial concepts are under consideration with various production capacities, it seems prudent to defer further discussion until further refinements have been developed.

The aforementioned irradiation facilities are being designed to help bridge the gap between laboratory results and commercialization, as well as provide radiation services to support food irradiation. Their development is a stated objective of the AEC program.

<u>U. S. Army Radiation Laboratory, Natick, Massachusetts (Fig. 7)</u>

This discussion would not be complete, however, without some reference to the forerunner of large food irradiation facilities—the U. S. Army Radiation Laboratory here at Natick. This facility, containing the largest known source

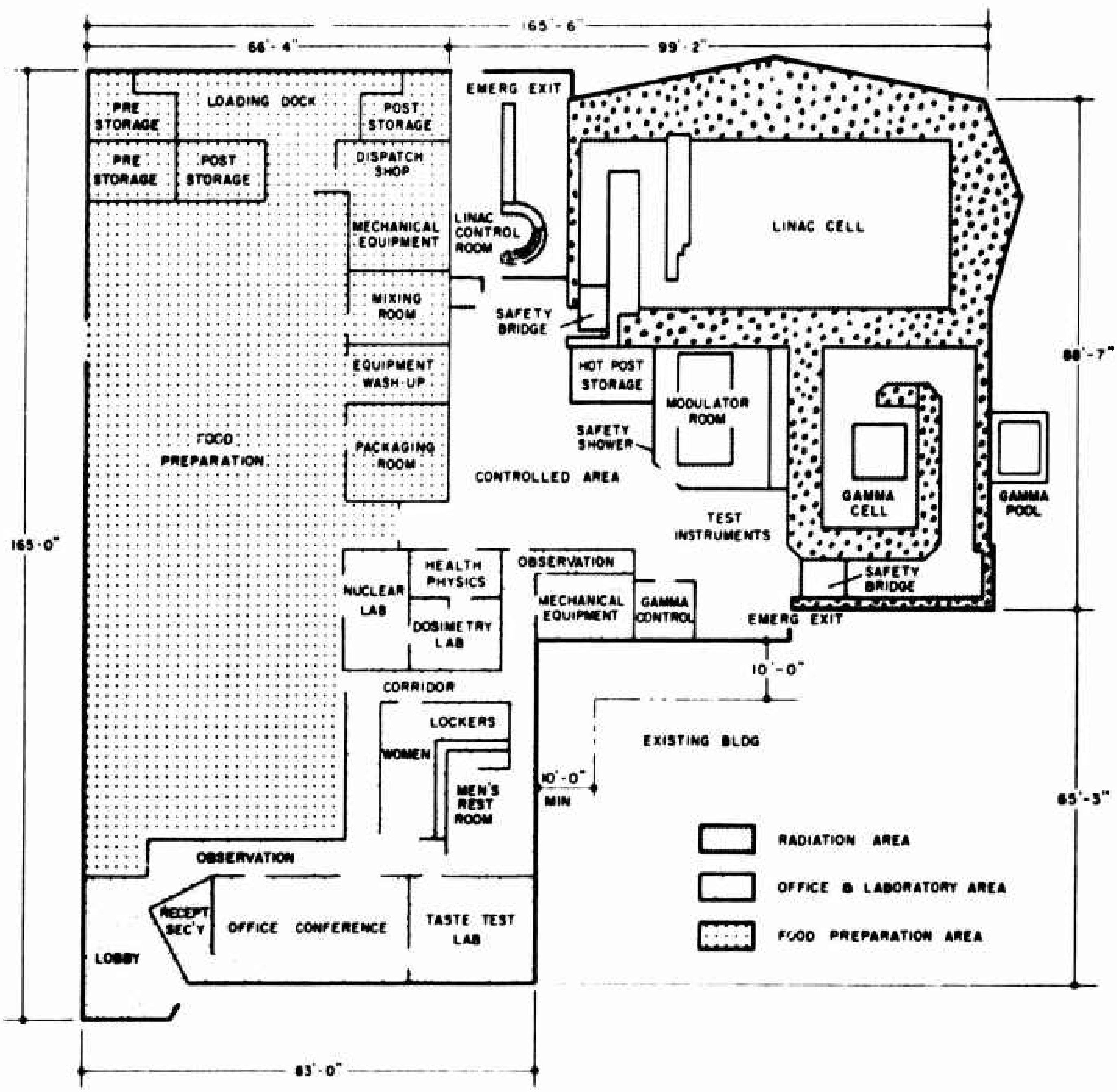


Figure 7. Radiation laboratory floor plan.

of cobalt-60, has been constructed primarily to support the Army's radiation sterilization of foods program. There are several unique features of this laboratory. The initial cobalt source of 1.3 million curies is the largest concentration of cobalt-60 used in one radiation source. A high energy 24-mev, 18-kw linear accelerator, expected to be fully operational in early 1964, is a second large radiation source within the same facility and will also be used to support food irradiation research. Finally, a large, complete food preparation area is contained within the building, thus providing a completely integral facility for food irradiation research. With precise controls built into the operation and functioning of the laboratory, this is an invaluable tool in furthering knowledge and capabilities in food irradiation. Associated Nucleonics, Inc., architect-engineer for the project, performed design services and supervised construction and test operation of the facility.

Conclusion

To conclude this presentation, several points are especially noteworthy. Since the clearance of radiation sterilized bacon by FDA, growing interest has been indicated by industry in food irradiation. This is especially so of those engineering firms which are leaders in the area of design and construction capabilities related to irradiation facilities. The initiative that some of these companies are taking with respect to encouraging the commercial use of food irradiation with food processors has been of tremendous value to the over-all program objectives. It is these engineering companies, in the final analysis, who must plan ahead, allowing months for design and construction, yet be able to furnish an appropriate facility when it is required by the food industry. I would like to acknowledge our thanks and appreciation for their efforts, and to encourage further participation along the lines already developed.

All of the designs presented herein consider only the use of cobalt-60 as the irradiation source. Its advantages are well known and its suitability for food irradiation has been accepted. There are, however, other sources which are expected to play a role in food processing, and they have been accepted by FDA in formal communication to be safe from an induced activity standpoint when used as food irradiation sources. Included is electron irradiation with electron energies not to exceed 10 mev and sealed gamma sources with gamma ray energies not to exceed 2.2 mev. The latter category includes such isotopes as cesium-137.

A third and final point is the consideration of costs of commercial irradiation facilities. Of course, actual construction costs represent the greatest expenditure of funds in any construction project. Engineering costs and cost of cobalt-60 also represent good percentages of total cost. There are several methods of reducing facility costs which should be considered in the development of a radiation facility. First, flexibility designed into a facility could be cut to the minimum if the facility were tailor-made for a particular size of package, or to process similarly packaged products. Secondly, until recently, the cost of cobalt in 100,000 curie lots has been one dollar per curie. The

AEC, on 20 September 1963, announced a price reduction in this category to fifty cents per curie. For a several hundred thousand curie source, here again is a substantial saving. Finally, the third area in which costs seemingly could be reduced is in the design engineering category. Standardization of sources, source holders, conveyors, and other equipment and construction features would eliminate special design costs, reducing them at most to adoption of standard equipment and design to the desired facility. Thus costs noted in the tables in this presentation should be considered as high compared to a commercial source since they are flexible in use and represent design efforts in a relatively new field.

The over-all conclusion to be drawn regarding radiation facilities is that engineering know-how and ability are sufficiently developed to assure that appropriate facilities can and will be built when the food industry and the consuming public are ready to utilize radiation processing.

ECONOMIC ASPECTS AND PROSPECTS OF COMMERCIALIZATION OF RADIATION PASTEURIZED FOODS

Joseph E. Machurek

In March, 1960, the AEC undertook the task of continuing the development of low dose radiation pasteurization of foods, with the objective of developing this technology to the point of both technical and economic feasibility. A number of fish and fruit items were selected, based on the Army's prior work, to be the most likely candidates for success.

Research during the past three years has resulted in the dropping of two of these items and the addition of others, primarily because further technology indicated mediocre response to radiation on behalf of those products which were dropped from the program. Continuing research, currently by AEC, and in the future by other Government agencies and industry, will identify increasing numbers of products which are amenable to radiation. Long term animal feeding studies will confirm their wholesomeness and safety. Commercialization, however, will be based on economics which, in spite of various marketing studies, can be most exactly determined only when the process is in existence at least on a pilot plant scale.

For the remainder of this presentation, I will cover economics and potential commercialization of radiation processed fish, fruits, onions, and potatoes and conclude with several comments pertinent to commercialization in general. These remarks are limited to the low dose or radiation pasteurization process.

A study conducted for the AEC by the U. S. Department of Interior, Bureau of Commercial Fisheries, dealt with the marketing feasibility of radiation pasteurized fishery products. Its purpose was to determine, from various segments of the fishing and allied industries, the acceptability and marketing feasibility of such sea foods. Personal interviews were conducted with over 300 producers, processors, distributors, wholesalers, retailers, home economists, and others. In general, the report showed the respondents to be quite enthusiastic, with minor reservations, and highlighted the following results:

a. Expansion of markets for fishery products ranked first among the expected advantages of radiation processing; improved quality control ranked second.

b. A cost range of 1/4¢ to 1¢ per pound, at the processors' level, was cited to be generally acceptable by about 40 per cent of the respondents. Thirty per cent of respondents indicated that a cost of 1¢ to 3¢ was acceptable, while a final 30 per cent would pay 2¢ to 5¢ per pound.

c. Results of the study indicate that many expect the radiation process to completely revolutionize the fresh fish industry, leading to the development of new markets and the expansion of old.

d. Extensive modification of present fish processing methods would not be required if the radiation process were commercially accepted.

Studies of facilities and processing costs indicates that initially we can expect to attain the 1¢ to 3¢ cost and, through further commercialization, facility refinement, and specialization, can eventually lower the cost to less than 1¢ per pound.

As far as commercialization is concerned, we must first obtain FDA clearance of the product. As soon as certain microbiological questions are resolved with the FDA, it is our intent to present a petition jointly with the Army for the clearance of cod and approximately ten other related bottom fish common to both the East and West Coast. The petition could be submitted this year if the FDA interprets our position relative to microbiology favorably. If, on the other hand, additional work is required in the Clostridium botulinum area, the petition could be delayed up to a year or so.

The low dose of 50,000 to 100,000 rads to control black spot in shrimp is also an exciting prospect for early commercialization, pendings its successful clearance by FDA. Not only is the fresh shrimp market expected to benefit by the radiation process, but interest has been indicated in the use of radiation to reduce bacterial counts prior to freeze dehydration.

Part of our program during the coming year will be devoted to large-scale processing, shipping, and storage of irradiated fish. The greater portion of these tests will be carried out by the USDI Gloucester Technological Laboratories utilizing the Marine Products Development Irradiator, while similar tests on other fish will be conducted by the contractors presently working with the particular specie. A case in point is Louisiana State University, which will conduct commercial size experiments with shrimp.

We are working closely with the National Fisheries Institute in the hope to bridge the gap to commercialization. The NFI, through various meetings and publications, is keeping their industry aware of the progress of this portion of our program and could prove to be the deciding factor when a commercial process becomes imminent.

Hence, in review, the technology data related to fish is nearing completion; the Army's long term feeding studies are virtually complete on cod,

which we hope to extrapolate to other fish within this class. Pending favorable interpretation of present microbiological data on fish, we will request FDA clearance for the above category of "bottom fish," with a target date of clearance in early fall, 1964, prior to the time the MPDI becomes operational. If large-scale tests bear out the very successful laboratory results, we would hope, through the USDI, the National Fisheries Institute, and others, to induce industry to attempt commercialization. This could be a reality in 1965.

Turning now to the related prospects for commercialization of radiation pasteurized fruits, it may be said that this phase of our program is both ahead-yet-behind the phase on fish. It is ahead in the respect that we have submitted, jointly with the Army, a petition to FDA requesting clearance of irradiated citrus. It is behind from the standpoint of over-all technology.

A sister study to the fish marketing study, previously discussed, has recently been completed on fruits. This study was conducted for us by the U. S. Department of Agriculture's Economic Research Service. Again, over 300 growers, shippers, produce wholesalers, and chain store produce managers were queried on radiation processing after they had been fully apprised of the program and its expected potential. Here also results were encouraging, and highlights included these general opinions:

a. The most often cited expected advantages of fruit irradiation were (1) reduced spoilage losses and (2) maintenance of quality. (Note that an extension of storage life *per se* was not specifically noted although this is inherent in an extension of quality.)

b. A majority felt that radiation processing would increase production and market volume of the selected fruit items, but would not affect output and sales volume of canned, frozen, and other processed forms.

c. Average costs per pound which respondents could pay for radiation processing depend on the type fruit. For instance, strawberries could bear a cost of 1-3/4¢ per pound, and other fruits surveyed—peaches, tomatoes, grapes, oranges, and grapefruit—could bear only 1/4¢ per pound.

d. Only minor changes in handling procedures would be required when radiation processing is incorporated.

Two conclusions may be drawn from the above and from work to date. First, chemical processing, where usable, is probably cheaper than irradiation. Secondly, radiation, where technologically appropriate, can maintain product quality in a manner equal to or better than chemical processing, and, further, with no chemical residue. In the case of citrus, presently preserved by the chemical biphenol, there appears to be no cost advantage to the use of radiation. Radiation, however, does purport to be a strong contender for citrus preservation in the event that chemical use was curtailed. It is also a strong contender where alternate preservation methods are unavailable.

Because technology related to fruits is not as advanced as that of fish, the area of large-scale shipping, storage, and testing is approximately a year behind that of fish. Included in this fiscal year's budget (FY 1964) are funds for the design and construction of a transportable irradiator, to be used in the fruit arm of our program. If funds are provided as requested, the unit could be completed in time to be utilized in the spring harvest of fruits in the California area in 1965. It is our present intent to follow this pattern of development.

Although semi-commercial testing is somewhat in the future, results of the Army's long term animal feeding studies are beginning to be forthcoming. We have tried to match feeding data of these products with development of technology data, and we feel that within the next few months there will be sufficient data to warrant submission of petitions to FDA for peaches and members of their related horticultural class, and probably for apples and pears. Thus we most likely will find ourselves in the position of having several fruits cleared for radiation pasteurization, but will not have shown a real economic advantage in their use. This would not, of course, preclude industry from commercializing the process if they felt it could be profitable. Ironically, the most technologically and economically promising product, strawberries, is one on which no past wholesomeness work exists. Accordingly, as Captain Whitehair noted, we plan to begin long term feeding of strawberries next spring as soon as the berries become available.

To summarize the economics and commercialization of fruits then, we find that, over-all, technology is behind that of fish. In certain cases, however sufficient data is available to suggest reasonable assurance of clearance by FDA. A petition requesting clearance of citrus is in the hands of FDA, and present plans include petitions for probably two other fruit classes yet this fiscal year. Where chemicals are presently used with success, there appears to be no clear-cut economic advantage in the use of radiation; radiation is, however, a good backup in this area and does offer distinct advantages where chemicals cannot be used. Commercialization could be forthcoming any time after FDA approval, even though we have not yet conducted large-scale testing or conclusively determined economics.

A common note of caution was indicated in both the USDI and USDA studies. Many of the respondents felt that before radiation could be successfully employed, an educational program to acquaint both industry and the consuming public with radiation would be required. Such a program would (a) acquaint the consumers with the radiation process, (b) inform them of its potential value, and (c) assure them that radiation processed foods are completely safe for human consumption. Realizing this need for public acquaintance with food irradiation, we have undertaken a study with the USDI to develop such an educational program. USDI has, in turn, agreed to implement the developed program, utilizing their existing network of public relations, news media, advertising, etc. The fully developed "Madison Avenue-type" program is expected within a year, with implementation soon thereafter.

A final comment regarding potatoes and onions is in order although these two items, because of doubtful economics, were not included in our original program. You may have noted in recent news articles that the Canadians have stated plans to incorporate radiation processing of potatoes into their economy this year. Southland Growers of Alberta, Canada, propose to build into a new processing facility a radiation capability for the purpose of irradiating onions, potatoes, and carrots. It is of interest to note that within the U. S. there is at least one organization also seriously and actively considering radiation processing of onions and potatoes beginning in the Fall of 1964.

A petition for clearance of potatoes is already in the hands of the FDA, and there appears to be a good possibility that onions may also attain clearance status in time for the 1964 harvest season. Obtaining this clearance is one of our primary and immediate goals.

In summary, the economic and commercial aspects of low dose food irradiation appear to be as follows:

a. The economics of fish irradiation are favorable. Nearly 60 per cent of the fishing industry indicated a radiation cost of 1¢ to 3¢ per pound could be tolerated. It is felt this cost can be met at present.

b. The economics of fruit irradiation are less favorable than for fish. Where chemicals are presently used and perform in a satisfactory manner, economics of fruit irradiation appear to offer no clear-cut advantage. Where chemical treatment is inadequate, such as for post-harvest storage of strawberries, irradiation does offer excellent results at an economically acceptable cost.

c. Several petitions are currently either in the hands of FDA for clearance of low-dose products or planned for submission in the next few months. With the clearance of a "base" of irradiated foods, it is felt that true commercialization could begin in the next two to three years.

d. Isolated commercial uses, such as for potatoes, could begin within the next year or two.

These then represent our current impressions of economics and potential commercialization of low-dose radiation processed foods. Radiation cannot be highlighted as a food preservation cure-all, but there is every reason to believe it can stand on its economic and preservation merits with a fair number of various products. For these reasons, we continue to be impressed and enthusiastic about its ultimate commercial success.

SESSION NO. 3

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WHOLESOMENESS OF FOODS PRESERVED BY COLD-STERILIZATION AND INDUCED RADIOACTIVITY

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Introduction

The various aspects which are related to the determination of the wholesomeness of irradiated food have been investigated since 1954 by the Medical Research Branch of The Surgeon General's Office (SGO). This broad program has been conducted through academic, commercial, and military laboratories as a part of the U. S. Army's interest in the application of ionizing rays for food preservation. Foods which are "cold-sterilized" by ionizing rays (gamma rays and electron beams) can be stored for long periods of time in essentially a fresh state without refrigeration. The advantages of such preserved foods in terms of storage, transportation, and troop morale are obvious.

Emphasis in the Army irradiated food program has been placed on sterilization; that is, the total inactivation of the normal microorganisms present. This requires a dose of about 4.5 Mrads. Low-dose irradiation or pasteurization requires doses of less than 1 Mrad. Recently, AEC has embarked on an extensive program to study the applicability of low-dose irradiation for increasing the shelf life of fruits, vegetables, and fish. Fortunately, the low-dose investigations will be able to draw upon the extensive data resulting from the Army investigations toward establishing wholesomeness and other studies.

This summary of SGO wholesomeness of irradiated foods will briefly review the over-all program up to the present time. Programs of the program since its beginning has been well documented in the scientific literature.[21,66,68,89,90,100] Over 30 different laboratories have participated in the wholesomeness studies and at the present time there are still several contracts actively engaged in the program.

While the greatest emphasis has been toward the establishment of wholesomeness (toxicity testing), areas such as nutritional adequacy, induced radioactivity, and other closely related areas have not been neglected.

Long-Term Studies

Since the greatest emphasis has been in the long-term feeding studies, they will be presented first. In Tables 1 and 2 are the general plans followed

TABLE 1

General Plan for Long-Term Wholesomeness Studies

Test Animals:	Rats, Dogs, Monkeys, Mice
Feeding Periods:	Two Years or Four Generations
Parameters:	Growth
	Reproduction[a]
	Hematology
	Longevity
	Histopathology
	Carcinogenicity

[a]Not studies in the mouse or long-term feeding of monkeys.

TABLE 2

Long-Term Studies
Two Years—Four Generations

Test Food:	35% (dry solids) in a nutritionally adequate diet
Irradiation Dose:	0, 2.79 and 5.58 Mrad
Irradiation Sources:	Spent fuel rods, Co^{60}, Electrons 1 and 2 mev
Rats:	25 males and 25 females per group
	First Generation (growth, reproduction and longevity
	First litter weaned and discarded
	Second generation weaned
	Second Generation (growth, reproduction)
	First litter weaned and discarded
	Third generation weaned
	Third Generation (growth, reproduction)
	First litter weaned and discarded
	Fourth generation weaned
Dogs:	2 males and 2 females per group
	Growth and reproduction for at least 2 years
	Pups observed for 8 weeks

in these studies.[36,56,57] Test foods were fed to two species of animals, usually rats and dogs for a period of two years or four generations, as 35 per cent (dry-solids) of a nutritionally adequate diet. Foods were irradiated with spent fuel rods, Co^{60}, or electron beams to 2.79 and 5.58 Mrads and stored at room temperature for at least three months before feeding. Non-irradiated control food was stored frozen. Each investigating laboratory

performed its own histopathologic observations and then submitted duplicate fixed tissue mounts to Armed Forces Institute of Pathology for review.[65,84,85]

Induced radioactivity should probably be included in the parameters studied under wholesomeness; however, since induced radioactivity could be studied apart from the feeding studies, it will be discussed under supporting studies.

Test foods for the long-term feeding studies are listed in Table 3. These were chosen as representative foods selected from the 40 foods cleared in short-term toxicity (8-12 weeks) rat-feeding tests.[3,69,70,92]

TABLE 3

Long Term Animal Feeding Studies
Radiation Processed Foods[a,b]

Food Tested	References	Food Tested	References
1. Bacon	27,46	12. Cole slaw	26,61
2. Beef, ground	5,6,14,37,43,75,77	13. Corn	44,58
3. Beef stew	17	14. Potatoes[e]	10,32,33,42
4. Chicken	6,80	15. Potatoes, sweet	1,56,57
5. Chicken stew	43,61	16. Fruit compote	35,46
6. Pork loin	7,9,43,97	17. Oranges[c,g]	4,59,60
7. Cod fish	1,56,57	18. Peaches[c]	4,96
8. Shrimp	22,23,59,101	19. Flour[f]	7,74,76,97
9. Tuna fish	43,58	20. Dried whole egg[d,h]	67
10. Beans, green	35,80	21. Jam	6,7,97
11. Carrots	7,22,23,95,97	22. Evaporated milk	18

[a]All foods irradiated to 2.79 and 5.58 Mrads except as indicated

[b]All foods were fed to rats and dogs except as indicated

[c]Fed to rats and monkeys

[d]Fed to dogs only

[e]Irradiated 7-40 Krads

[f]Irradiated 37 and 74 Krads

[g]Irradiated 140 and 280 Krads

[h]Irradiated 0.9 and 2.79 Mrads

In the following discussion of the long-term feeding studies, only those results which are of interest will be presented. If a food is not discussed, it is to be assumed that there were no significant differences between the control and irradiated diet parameter findings.

Irradiated beef fed dogs were reported by McKay[43] to have decreased reproduction performance. One of the contributing factors was thought to be a submarginal vitamin E deficiency. In order to investigate this further, two

repeat experiments, with adequate vitamin E supplementation, have been in progress for almost three years by Clarkson[14] and Loosli.[37] Results to date do not show decreased reproduction performance in the dogs on the irradiated beef diet. It is of interest that Clarkson reported that the bitches on irradiated beef had their first estrus significantly sooner than those on control diet. Loosli, on the other hand, reported no significant differences in days to first estrus.

Recent reports by Watson[101] have made reference to a high incidence of thyroiditis in dogs eating aged (3-5 years) irradiated shrimp. This finding is still being studied by Watson. Reber had previously reported a questionable increased incidence of thyroiditis in dogs fed irradiated flour. Since thyroiditis has been observed by others in dogs on non-irradiated diets, Watson[101] has studied the thyroids from 100 dogs obtained from a local dog pound. A 27 per cent incidence of thyroid lesions, similar to thyroiditis, was found. The AFIP in a review of the 270 dogs in the wholesomeness studies concluded that while an over-all incidence of thyroiditis was about 16 per cent, there were no significant differences attributable to either food or level of irradiation.[86A,98]

Irradiated carrots were reported by Tinsley[97] to decrease growth rate and carotene utilization in rats. There was reason to believe from the poor condition of the irradiated carrots that they were contaminated with a resistant microorganism. This study is being repeated. No decrease in growth or carotene utilization was observed with frozen stored irradiated carrots. Therefore, it may be reasonably assumed that irradiation per se is not detrimental. Repeat feeding of room temperature stored carrots only slightly reduced growth and carotene utilization. While these irradiated carrots are a better product than those used in the first rat study, there is reason to believe that they are still contaminated.

In five long-term rat-feeding studies, two irradiated foods (0, 2.79, and 5.58 Mrads) were each fed simultaneously at 35 per cent of dietary solids in a 3 x 3 factorial design to determine any interactions. These more complex studies were negative in regard to the several combinations of food and level of irradiation as reflected by the parameters studied.

With the exception of the doubtful findings of thyroiditis and decreased growth with irradiated carrots, no other adverse findings were reported.

The next series of experiments, Table 4, are the studies which specifically investigated the possible carcinogenicity of irradiated foods. For the carcinogenicity studies, mice were utilized since they are more susceptible to carcinogens than rats. Two studies used composite diets of five or six irradiated foods furnishing 100 per cent of the total caloric intake. One study utilized 20 per cent bacon lipid rendered from irradiated bacon. Sterols when irradiated have been reported to form carcinogens. To investigate this possibility, extracts of irradiated foods high in sterols as well as pork brain and egg sterol concentrates were painted and injected. No significant differences in lesion or tumor incidence between the irradiated and non-irradiated diets or extracts were observed in mice or rats.

TABLE 4

Carcinogenicity Studies
Radiation Processed Food

A. Composite Diet, 100 per cent irradiated, 5.58 Mrad, Mice

Study 1 (12)		Study 2 (19)	
Cod fish	Beef stew	Beef	Sweet potatoes
Green beans	Peaches	Corn	Fruit compote
Chicken stew	Flour	Tuna fish	

B. One or two irradiated diet components

1. Bacon lipid, mice (20)
2. All long-term feeding studies (see Table 3)

C. Sterol Concentrates, Mice and Rats (31)

Pork brain	Cheese
Egg	Fish
Vegetable oils	Lard
Meat	Milk

Extracts painted, injected and fed

Carcinogenicity and tumor incidence were also investigated in the long-term feeding studies. It was mentioned above that AFIP review duplicate sets of fixed tissues from the various contractors in the program. The AFIP individual reports have been completed and their findings concur with those of the individual contactors. Conclusions are that there have been no consistent findings which can be attributed to either food or level of irradiation. A final report incorporating a summary of all tissues reviewed from the wholesomeness program will be submitted in the near future.

Special Studies

Two special studies are listed in Table 5. The first is a long-term rat-feeding study in which a nine component composite diet supplied 100 per cent of the total caloric intake. No significant differences in the parameters studied were found between the irradiated or control diets.

The second study was designed to investigate the degenerative heart lesion in mice which was first reported by Monsen.[52,53] During the course of a mouse carcinogenicity study with a five component 100 per cent irradiated diet, a high mortality rate was observed, particularly in those mice eating the irradiated food. The heart lesion which was contributing to the high mortality

TABLE 5

Special Studies
Radiation Processed Foods

A. Long-term rat feeding study with a nine component, 100 per cent irradiated* diet.[72]

Bacon	Beets
Beef	Peaches
Ham	Cereal bar
Beans	Milk, powdered whole
Haddock	*5.58 Mrads

B. Degenerative heart lesion in mice [52,53,94]

1. All five diet components irradiated to 5.58 Mrads
2. One component irradiated
3. Diet cooked or uncooked
4. With or without added vitamins
5. Milk only dietary component
   (Twenty-eight different combinations)

pork	carrots
chicken	evaporated milk
potatoes	

rate was subsequently observed by Monsen in mice fed non-irradiated evaporated milk diets. The USAMRNL comprehensive study, Table 5B, utilizing over 4,000 mice and detailed histopathologic examinations has been completed.[94] A final report of this study is being prepared for submission to OTSG. The mice used in this study were originally obtained from Monsen's stock colony. Conclusions of this special study are: 1) That no "Monsen type" heart lesions were observed in any phase of the study, and 2) that other lesions were found with equal frequency in both irradiated and non-irradiated diet groups.

Human Feeding Studies

As early as 1957-58, short-term (2-week) feeding studies with human volunteers, Table 6, were conducted at USAMRNL. Seven separate feeding tests were carried out, each with 9 or 10 test subjects with 54 foods irradiated from 9.3 Krad to 3.72 Mrad stored frozen or at room temperature for three months. Irradiated foods were fed up to 100 per cent of the total daily caloric intake. There was no evidence of toxic effects in any of the men either by clinical or laboratory tests. Digestibilities of the macronutrients and metabolizable energy were similar in the irradiated and non-irradiated diets.

Human Feeding Studies

1. Short-term (two weeks)[45,62]	54 foods irradiated up to 3.72 Mrad fed during seven feeding studies one of which utilized a 100 per cent irradiated diet
2. Troop Acceptability[11]	One irradiated component in a standard menu meal

Also, starting in 1958, Table 6, studies on the acceptability of irradiated foods have been conducted at Fort Lee, Virginia. In these studies, one irradiated food was included in one standard mess meal and was served to volunteer troops. The several irradiated foods tested have been as acceptable as their respective standard non-irradiated food.

Supporting Studies

In Table 7 are listed the supporting studies which have been conducted in areas of importance to the over-all interest in the wholesomeness of irradiated foods.

TABLE 7

Supporting Studies for the Wholesomeness Testing Program

Studies	References
1. Induced Radioactivity	25,48,88,91
2. Nutrient Stability 3. Nutritional Adequacy	2,8,13,15,16,30,49,55,79,81,82,93,99,100,102
4. Tissue Enzyme	8,54,55,78,95
5. Digestibility	2,54,55,62,87
6. Vitamin K Nutrition	21,28,29,38,39,40,41,47,50,71,73,86
7. Chemical Changes	2,30,54

1. Induced radioactivity. There has been an increasing awareness to radioactivity and its effects on materials and living organisms. This has been due in large part to the much publicized fallout from nuclear explosions. Equally important is the increased practical application of radionuclides in

industry, medicine, and research. Sterilization of foods by ionizing rays is one of these applications. In this application the awareness of radioactivity is illustrated by the fact that the question, "Are irradiated foods radioactive?" is asked more often than, "Are irradiated foods wholesome?"

The answer to the question on radioactivity is that there is no detectable induced radioactivity in foods irradiated to 5 Mrads with gamma rays or electron beams having energies of less than 10 mev. While this statement is supported by experimental evidence and theoretical considerations, it is of interest to list the variables which must be considered in the production and detection of induced radioactivity in foods.

a. Irradiation source
b. Type of radiation
c. Energy of radiation
d. Per cent abundance of target element
e. Cross-section of target
f. Half-life of produced radionuclide
g. Detector sensitivity
h. Radioactivity from natural and fallout sources
i. Container
j. Contamination from source

A discussion of the above variables is not within the scope of this paper; however, brief mention will be made of some of them in reviewing the data presented by Stanford Research Institute[25,88,91] (Table 8) and Meneely[48] (Table 9).

Stanford Research Institute (SRI) in a very comprehensive investigation of induced radioactivity studied (1) the irradiation of elements in foods expected to produce radioactive products through the low energy requiring isomer activation (γ, γ^1) reaction and the higher energy requiring particle emission reactions, (2) the irradiation of foods enriched with these elements, and (3) the irradiation of unenriched foods. Sources employed were Cs-137, Co^{60}, spent fuel rods, as well as electrons and X-rays with energies of 4-25 mev.

From these studies it was determined that:

a. Induced isomer activity is experimentally undectable in foods. The calculated radioactivities of Sn-117m and Sn-119m, relatively the most important, in foods irradiated with 24 mev X-rays are at least one order below the natural H-3 radioactivity.

b. In beef irradiated with 24 mev electrons, it was calculated that nine radionuclides with half-lives of longer than 10 days could be found. Of these, seven could be detected only if the food element was enriched before irradiation or concentrated after irradiation. Only two elements, Na-22 and Rb-84, could be detected directly. Of the several samples of beef tested in the various phases of this study, Rb-84 was found in only one sample.

Radioactivity in Food
(micro micro curies/gram)

Natural		
K-40	2.0	
C-14	1.5	
H-3	0.001	
Ra-226		
Fallout		
Cs-137	0.16	
Sr-90	0.001	
Zn-65	0.02	
Detectable, Induced (SRI)[25,88,91]		
Na-24	10 mev	0.02
	12 mev	0.5
Na-22	14 mev	0.004
Rb-84	24 mev	0.05

TABLE 9

Induced Radioactivity
(Meneely[48])

	Not Detectable			Detectable
Pork	Co^{60}	8 mev	11.2	12 13 16 pork
Chicken				Sc-46, 47, 48
Beef	Co^{60}	8 mev	----	Mn-54, Na-24, Xe-133m
Ham				
Bacon	Co^{60}	-----	----	P-32

c. In a study where six foods and a composite diet were irradiated to 5 Mrads with electrons, only one induced radionuclide (Na-24) was detected at energies less than 12 mev. The short-lived Na-24 was detected at 12 mev in ham, chicken, and shrimp and at 10 mev in the composite diet. The long-lived Na-22 was detected at energies greater than 14 mev. At 14 mev, the Na-24 and Na-22 produced were present at concentrations of 10^{-3} and 10^{-5}, respectively, below the maximum permissible concentration in water. No beta emitters were detected.

Studies by Meneely showed no detectable induced radioactivity in foods irradiated with Co^{60} or electron beams of less than 11.2 mev. At energies between 12-16 mev, six gamma emitters and one beta emitter (P-32) were detected.

From these two investigations, it was concluded that there is no detectable induced radioactivity in foods irradiated with electron beams of less than 10 mev. Of the nine radionuclides identified and detected at energies greater than 11.2 mev, only five had half-lives greater than 10 days, Mn-54, Na-22, Rb-84, Sc-46 and P-32.

To illustrate the magnitude of induced radioactivity in foods, it was calculated by SRI that the body burden of ingesting 100 per cent of one's diet of 24 mev electron irradiated food would be 0.26 mr/year. This includes all radioactive elements produced whether or not they are detectable. (This figure of 0.26 mr/year is probably a high estimate since most of the calculated values based on enriched samples tended to be high by at least a factor of 2.) The body burden and external irradiation from natural sources has been estimated to be about 150 mr/year of which 5 mr is contributed by fallout products.

The significance of the statement "no detectable activity" can be appreciated when one considers that the gamma counters used can detect 0.001 $\mu\mu$ curie/gram of food or, in other words, several nuclei out of 10^{25} nuclei. This sensitivity is incomparably greater than any chemical methods of detection. In spite of the relatively small amount of radioactivity induced by 25 mev electrons compared to natural sources, irradiated foods are still subject to the Pure Food Law, "Food Additives Amendment" of 1958 under the "Delaney Cancer Provision." In effect, this amendment requires a "zero" tolerance of substances added to foods which have been shown to be carcinogenic in any concentration. This includes radionuclides.

The interpretation and application of the "Delaney Cancer Provision" in regard to irradiated foods is made difficult by the lack of definitive data on the effects of added radionuclides in $\mu\mu$ curie levels on the biological mechanism. In this connection, the following quote from the Federal Register illustrates the awareness of the problem:

> "Fundamentally, setting basic protection standards involves passing judgment on the extent of the possible health hazard society is willing to accept in order to realize the known benefits of radiation. It involves inevitably a balancing between total health protection, which might require foregoing any activities increasing exposure to radiation, and the vigorous promotion of the use of radiation and atomic energy in order to achieve optimum benefits."[24]

2. Nutrient stability. It has been adequately shown that irradiation of foods does not cause any unusually significant destruction of nutrients that does not occur in conventional heat-processed foods.

3. Nutritional adequacy. The nutritional adequacy of irradiated foods has been amply demonstrated by the long-term feeding studies. In none of these studies has there been found an increased requirement for known micro or macro-nutrients in normal or stressed situations. Some of the diet components in Table 3 undoubtedly were a stress to the animals.

4. Tissue enzymes. In general, no significant changes in tissue enzyme activity have been demonstrated in animals eating irradiated foods if the diets are supplemented with normal amounts of required vitamins. A few short-term *in vivo* and *in vitro* experiments have shown both a depressed or elevated activity of tissue enzymes. Unfortunately, too little is known of the significance of enzyme rate changes to the over-all functioning of an organism to be adequately interpreted. Enzymes adapt readily to changing environments in order to adequately meet the needs of the moment. Therefore, change in itself is not detrimental. The effect of the change can best be evaluated by the performance of the whole animal over a long period of time as in the long-term feeding studies.

5. Digestibility. Several *in vivo* and *in vitro* studies of the digestibility of various irradiated food components have been reported. In general, the short-term studies, both *in vivo* and *in vitro*, do show decreased rates of digestion. An illustration of the short-term *in vivo* experiment was that reported by Schreiber and Nasset.[87] In this study, lard or lard-meat mixtures were fed to dogs by intubation. After three hours, the animals were sacrificed and stomach and intestinal contents were analyzed. The irradiated (5.58 Mrad) lard was retained in the stomach to a greater extent than the non-irradiated lard (65 vs. 49%), filled the small intestine to a lesser extent (9 vs. 13%), and was absorbed to a lesser extent (26 vs. 38%). Less absorption was also indicated from jejunal fistula collections over a 12-hour period. Attention should be made to the lard used in these experiments. The initial peroxide number (PN) of the irradiated lard was 176 and continued to rise to over twice this value over a period of 14 months in cold storage. Normally, irradiated fats have peroxide numbers of less than 100.

Oxidized soy oil with a PN of 100 or less gave normal growth and reproduction when fed at 20 per cent level in diet.[2] No differences were found in chick growth between irradiated or non-irradiated corn or soy oil; however, poor growth and high mortality was shown in chicks fed irradiated pork or beef fat and non-irradiated pork fat.[83] Supplementation of these fats with anti-oxidants or vitamins A and D resulted in normal growth. Carbonyls were found to depress *in vitro* lipase activity and *in vivo* rate of fat absorption, but had no effect on net utilization over a 20-day feeding period in rats.[54]

Moore, while reporting a significant reduction in the digestibility of irradiated corn oil over an 8-month period, made the following statement "...it is doubtful whether the gain or loss of digested nutrients would be of biological significance."[55]

While it has been demonstrated that the rate of fat digestion may be decreased, the short-term, long-term, and carcinogenicity studies have demonstrated that net utilization is not affected significantly. As in the case of enzyme activity, there is no evidence to indicate the physiological significance of the findings of the short-term digestion experiments. This aspect deserves further investigation, although it is not a wholesomeness problem.

6. Vitamin K nutrition. The most fruitful development from the wholesomeness studies has been in advancing our knowledge of vitamin K nutrition. A vitamin K nutritional requirement was first described in rats with an irradiated beef diet. It was soon discovered, however, that non-irradiated pork or soy protein diets could produce even more severe symptoms of a vitamin K deficiency in rats. In every case the deficiency was corrected by physiological doses of vitamin K. As these studies developed, it soon become obvious that vitamin K nutrition and metabolism was influenced by the following factors:

a. Protein sources
b. Amino acids
c. Coprophagy
d. Strain, age and sex
e. Species
f. Vitamins A and E
g. Bile acids
h. Lipids
i. Aldehydes

7. Chemical changes. Only limited data have been obtained in the wholesomeness program on the chemical changes in foods which are peculiar to irradiation. These data have almost been limited to the oxidative changes of lipids. As in the case of tissue enzymes and digestion rates, the significance of these changes could not be determined.

Summary and Conclusions

In summary, it can be stated that foods irradiated to 5.58 Mrads are wholesome and their nutritional adequacy is comparable to conventional heat-processed foods. The findings which at first seemed to implicate irradiated foods are given in Table 10. While the aged shrimp-thyroiditis relationship is still under study, evidence has been submitted to show (1) that pound dogs have a high incidence of spontaneous lesions similar to thyroiditis and (2) that in the 270 dogs from the wholesomeness studies neither food nor level of irradiation could be related to thyroiditis. The decreased digestion rate of irradiated lard is not a wholesomeness problem. This has been demonstrated in the long-term feeding studies where net utilization was not influenced when high fat foods were fed. The decreased growth rate of rats on an irradiated carrot diet appears not to be due to irradiation per se, but probably to

inadequate sterilization. Studies on the irradiated carrot problem are still in progress. The heart lesion studies have been completed, and it has been demonstrated that irradiation is not the causative factor.

The present status of radiation processed foods in regard to FDA clearance and utilization is given in Table 11. Since January 1963, clearances have been obtained for canned bacon irradiated to 4.5 Mrads with Co-60 or 5 mev electrons and wheat, including wheat products, irradiated to low doses. Two petitions, potatoes (low dose) and bacon irradiated with 10 mev electrons, are still pending. No large-scale utilization of irradiated foods or long-term human feeding tests have been initiated.

TABLE 10

Review of Findings
Radiation Processed Foods

Food	Animal	Observation	Investigator
Flour, shrimp[a]	Dog	Thyroiditis (?)	Reber, Watson[a]
Lard	Dog	Digestion rate (N)	Nasset
Carrots[a]	Rat	Growth rate (N)	Tinsley
Composite Diet	Mouse	Heart lesion (N)	Monsen; MRNL

[a]Studies still in progress.
N = Not a wholesomeness problem or related to irradiation.

TABLE 11

Present Status of Radiation Processed Foods
and Clearance by FDA (Sept 1963)

1.	Bacon	Co-60	4.5 Mrads
2.	Bacon	5 mev	4.5 Mrads
3.	Wheat and wheat products (low dose)		
4.	Potatoes (pending)		
5.	Bacon	10 mev (pending)	
6.	Large scale utilization or long-term human feeding studies (none)		

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PRESENT STATUS OF THE WHOLESOMENESS PROGRAM

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The irradiated food program represents man's greatest effort toward the practical application of one method for food preservation. After 10 years' intensive study, irradiated foods may not be as well understood as we would like, but we have gained a tremendous amount of information from the volumes of data resulting from these studies. The wholesomeness studies in themselves represent a heroic effort toward the establishment of the non-toxicity and nutritional adequacy of irradiation processed foods. One is led to wonder what the results would have been if all of our present day foods and processes had been viewed as critically.

In reviewing the progress of the wholesomeness program over the years, it seems that the many favorable findings have been subordinated to the few dramatic findings which have caused temporary alarm, such as reproductive failure, hemorrhagic syndrome, and heart lesion. The unfavorable results have been explained and corrected simply by increasing our nutritional awareness or demonstrating that the finding was not peculiar to radiation processed food.

The point of this is that there was, and perhaps still is, a certain amount of preconditioning to the hazards of radiation. Unusual results were expected and a few were found. It is believed, however, that in the final analysis radiation processed foods will be found to be as wholesome and adequate as the conventionally processed foods we have been accepting without question in our daily diet.

Frequently, regarding wholesomeness it is asked, "Where are we now and where are we going?"

The first part of this question (where are we now?) has almost been answered satisfactorily. The wholesomeness and nutritional adequacy of most foods irradiated within the limits of the goals established in 1953 have been demonstrated quite adequately. At that time the interest was in canned foods, treated with radiation energies of about 1 mev and a total dose of 5.58 Mrad.

The second part of the question (where are we going?) is not as easily answered because it depends not only on what is desired, but, more important, whether or not the desired technical goal will be acceptable after it is achieved. Our problem stems from the advancement of radiation technology, economic factors, and the lack of information on the effects of small amounts of ingested

radionuclides. Economic considerations favor the use of high power high energy electron (100 watt, 24 mev) accelerators over the relatively inefficient and scarce Co^{60}; however, our present food safety laws prohibit the use of such high energies because of the measurable, though tiny, amounts of radionuclides produced.

Our problem can be further defined by asking whether we are satisfied with our present limits or do we desire higher energies and new packaging materials. It thus becomes apparent that the 1953 goals should be re-evaluated in terms of the knowledge gained since that time. One result of the re-evaluation should be a new set of limits within which we can reasonably operate. In establishing the new set of limits, the problem of induced radioactivity and wholesomeness should be given serious consideration if energies of 10-11 mev are to be exceeded. The difficulty lies in the fact that our present criteria for wholesomeness may not be acceptable if any induced radioactivity can be demonstrated.

At USAMRNL we have started a series of studies in cooperation with the Natick Radiation Laboratory to study chemical and nutrient changes in foods irradiated with 24 mev electrons. Bacon, the first food sample received, was irradiated with 18-24, 17, 11 mev and Co^{60} to 5 Mrads. All samples including controls have been stored frozen. Room temperature storage will also be studied. The purpose of this investigation is to determine changes in lipids, protein, vitamins, and other nutrients as they may relate to dose rate and energy of radiation. Sterility of the irradiated samples and induced radioactivity will also be measured. There is very little information on the comparative effects of different radiations and dose rates on uniformly prepared and treated foods. The results of this study should be of some assistance in the preliminary evaluation of the wholesomeness of foods irradiated with high energy electrons and the establishment of the upper limits of radiation energy.

Data obtained after a limited number of different determinations have not shown any differences between the control bacon and bacon irradiated with different energies.

Other investigations which are currently in progress under OTSG contract are:

1. Further studies on induced radioactivity in electron irradiated meats.

2. Study of growth rate differences in animals fed irradiated beef. Consistently, though not significantly different, growth differences seem to be greater between the two levels of irradiation (2.79 and 5.58 Mrads) than between either irradiated dose and non-irradiated control.

3. Study of irradiated polyunsaturated fatty acids. Ultraviolet radiation produces in polyunsaturated fatty acids a water-soluble product which is highly toxic to mice when injected intraperitoneally. The nature of the product(s) formed is being investigated.

4. Other projects in progress:

a. Beef - Dog reproduction
b. Carrots - Rat growth
c. Shrimp - Dog thyroiditis
d. Composite diet - Mouse heart lesion
e. Vitamin K nutrition and metabolism
f. Histopathology of the long-term feeding studies

Conclusions and Summary

It is felt that the wholesomeness of foods irradiated with 1-2 mev sources to 5.58 Mrads has been adequately demonstrated. As these studies are nearing completion, it is suggested that further work with irradiated foods should be evaluated on the basis of experience and knowledge gained from these and other studies. The use of higher energy electron beams seem to offer many technical advantages; however, with the use of such sources new criteria for the establishment of wholesomeness may be required

SESSION NO. 4

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PRODUCT DEVELOPMENT

Fred Heiligman

Introduction

At the last Contractors' Meeting in 1961, the status of the Product Development Program and problems associated with it were presented. Since then some progress has been made in the solution of these problems, but they are still far from being solved. The changes in sensory characteristics associated with irradiation of many foods are still among the most formidable problems requiring solution. The information reported here is the result of studies conducted by many academic, commercial, research, and governmental agencies in cooperation with the Food Division of the U. S. Army Natick Laboratories and from studies conducted by the Irradiated Food Products Branch of the Food Division.

For convenience, the research in the product development area has been divided in three broad areas: pre-irradiation, concurrent-irradiation, and post-irradiation. In the pre-irradiation area, studies are being done to determine the influence of variations in factors that can be controlled prior to irradiation. Among these are type and quality of the raw material, method and extent of enzyme inactivation, the use of selected additives and odor and oxygen scavengers, and packaging environment. In the concurrent-irradiation area, such factors as variations in total dose, dose rate, and product temperature during irradiation are studied to determine their influence on product quality and storage stability. And, in the post-irradiation area, studies are being done to develop recipes and optimum cooking methods for using irradiated meats and to determine the acceptability and storage stability of the products. Efforts are being concentrated on meat items (beef, pork, ham, chicken, and fish).

Pre-Irradiation Studies

Enzyme Inactivation

Long term storage of irradiated foods at nonrefrigerated temperatures requires the destruction of the autolytic enzymes. This cannot be achieved at irradiation dose levels as low as the levels of radiation required for bacterial control. Currently, enzyme inactivation is being done by heating the foods as this is the only satisfactory method known to date. The results of many studies on many kinds of meats (beef, pork, fish, and chicken) show

that internal temperatures of 160°-170°F are required to completely inhibit proteolysis of the meats while they are in storage. Heating to these temperatures essentially cook the meats; thus, when they are reheated for serving they have warmed-over flavors and are frequently dry and friable. If they are eaten cold, they have typical cold cooked meat flavors.

Experimentation has shown that enzyme inactivation by methods requiring the shortest periods of time to reach the desired internal temperature usually yield a product that has better textural characteristics. Enzyme inactivation of meat items by methods using short periods of time to obtain the desired internal temperatures, as contrasted to similar products prepared by using longer periods of time, are juicier, firmer, and less friable. For example irradiated beef roasts that had been enzyme inactivated in 350°F dry heat ovens were preferred to roasts that were enzyme inactivated in 185°F smoke house or in steam at atmospheric pressure. This difference in preference is thought to be due to the differences in the texture of the products. Results of this nature have also been obtained with both roast pork loins and chicken parts.

Type and Quality of Raw Material

The type and quality of the raw material used in preparing the irradiated meat products have a marked effect on the quality and storage stability of the products. Variation in these factors have been shown to influence the degree of radiation induced changes in texture, flavor, and other sensory characteristics.

Low grades of beef such as U. S. Commercial, a product high in connective tissue, usually yields an irradiated product that is preferred to a similar item made from high grade beef, such as U. S. Choice which is low in connective tissue. This preference for the products made from the lower grades of meat seems to be related to the combined action of the heat used for enzyme inactivation and of the irradiation on connective tissue. The combined treatments have over-tenderized the meat low in connective tissue as the meats are usually soft, mushy, and friable. Whereas, in products made from low grades, which would normally be tough, the combined treatments of heat and irradiation have produced a good textured product. Irradiation may provide an excellent means for up-grading low cuts of meats because of this tenderizing effect.

The amount and degree of intramuscular fat in raw pork loins has been studied to determine their influence in the acceptability and storage stability in irradiated roast pork loin. These factors did not seem to influence the acceptability of the irradiated product when served as hot roast pork, but when used to prepare cold pork sandwiches, the products low in fat were preferred. High degrees of marbling combined with radiation resulted in greater tenderness as determined by mechanical means. It is interesting to note that irradiated samples had lower TBA values than comparable non-irradiated samples, but the degree of marbling seemed to have no effect on the TBA values.

Studies on the acceptability of irradiated cured meats have shown that there is a wide variation in similar cured meat products prepared by different commercial companies. This variation seems to be associated with the curing. The factors causing these variations are not known but are being investigated. When this information is available, the types and quality of cured meats needed to consistently produce high quality shelf-stable irradiated cured meat products can be developed.

Only a limited amount of work has been done to determine the type of raw chicken best suited to produce irradiated chicken parts Older broilers produce an irradiated product with less friable bones than younger broilers. Irradiated breasts and thighs from 3 to 3-1/2 pound broilers seem to be preferred to similar parts from 4 to 5 pound fowl; and, as expected, acceptance scores for both irradiated and non-irradiated white meat are higher scores than for the dark meat.

Additives

Attempts to suppress irradiation flavor through the use of additives have not been too successful. The skillful use of spices and condiments and the adaption of appropriate culinary practices in preparing the meats has resulted in increased acceptance in some products.

Sauce and spice combinations have been developed which seem to improve the acceptability of beef. The most successful have been barbecue sauce, beef and mushroom sauce, pepper and tomato sauce, and ascorbic acid and onions in beef sauce. Whether these sauces and spices mask irradiation flavor by alteration in the chemistry of the products, or merely cover up irradiation flavor is not known at this time. In any event, it must be pointed out that these products are in a different class of products than those that require typical beef flavor.

The influence of a large variety of tomato products (juice from several varieties of tomatoes, tomato sauce, tomato paste, and tomato serum) on irradiation flavor intensity and typical beef flavor in irradiated beef has been studied. The results did not show any beneficial effect by adding tomato to the beef prior to irradiation; however, after irradiation, addition of tomato during preparation for serving did seem to reduce irradiation flavor intensity and improve acceptance of the products.

As earlier mentioned, differences in curing methods result in differences in acceptance scores and in the irradiation flavor intensity in hams and bacon. High salt concentrations do not reduce irradiation flavors or improve the acceptance scores of either irradiated ham or bacon. There are suggestions that surgar curing and sugar glazing improve the acceptability of irradiated hams.

No beneficial value has been shown to be attributable to the addition of antioxidants in either beef or pork items. Certain antioxidants have been shown to be effective in increasing the storage stability of irradiated codfish, but these materials did not seem to increase the stability of irradiated halibut.

Scavengers and Packaging Environment

Continued observations have confirmed earlier reports that the addition of activated charcoal to the sealed container reduces irradiation odors in the head space gases. Additional acceptance studies have also confirmed previous results that the addition of charcoal improves the scores of both irradiated pork and chicken. At the levels of activated charcoal used in these studies, which was 0 to 2 per cent, no beneficial effects could be observed in improving the acceptance of beef steaks. In other experiments, in which up to 25 per cent of the package volume was filled with activated charcoal, the treatments were extremely effective. In every experiment, which included ham, bacon, chicken, and beef, irradiation odors were drastically reduced and were barely detectable in the cans that contained 25 per cent active carbon.

No beneficial effects have been noted in foods packed with in-package oxygen scavengers of the glucose-glucose-oxidase-types of scavengers. High vacuum packaging appears to be as effective as this type of treatment.

Studies involving packaging environment continue to point out the need for vacuum packaging. In one series of studies in which low temperature (-320°F) irradiated beef steaks were packaged in these different environments, the steaks packed under vacuum had less irradiation flavor and were more acceptable than the steaks packed with nitrogen and oxygen back fill.

The objectionable pink color, frequently associated with irradiated chicken packed under vacuum, can be partially eliminated by enzyme inactivation to slightly higher internal temperatures (185°F); thus the objectionable odors that have been associated with air packed chicken seem to be reduced and the off-color partially eliminated.

Some recent observations have been made in flexible packaged hams. It seems that when metalized plastics and high vacuum were used, rancidity and other objectionable characteristics were slower in developing than comparable hams packed in clear plastic under lower vacuum. No conclusions can be made at this time as more work is needed in this area.

Vacuum packaging also improves the storage stability of fish products. Codfish cakes packed under vacuum were much preferred to air packed cakes. This preference was observed shortly after irradiation and after six months storage at 70°F.

Concurrent Irradiation Studies

Influence of Total Dose

Studies designed to determine the influence of irradiation doses of less than 4.5 Mrad on product quality have been started. Data to date are meager but, as anticipated, irradiation at lower doses improves the acceptability of the products. Hams and bacon that have been irradiated at 2.5 Mrad, a dose which seems to be reasonable for cured meats based on recent microbiological data, are more acceptable than the same products treated with 4.5 Mrad.

Product Temperature

In all experiments in which the product temperature during irradiation is below freezing, the quality of the irradiated product seems to be improved. This has been observed in chicken, beef, turkey, hams, and fish.

Considerable research has been done on irradiation of beef over a wide range of temperatures, and the results show that radiation flavor intensity decreases as product temperature during irradiation decreases. This relationship seems to be linear in the range studied (-320° to 80°F). Slow warming after irradiation at -320°F or holding the product at low temperatures after irradiation is reported to reduce radiation induced changes, as contrasted to warming at room temperatures immediately after irradiation.

Acceptance studies on beef that were frozen to -60°F and irradiated without additional efforts to hold it at this temperature showed a marked reduction in irradiation flavor and an increase in over-all acceptance. It required slightly over one hour to irradiate the beef; the product remained in the frozen state during irradiation. Similar results have been obtained with hams; however, in this product, irradiation at subzero temperatures seem to adversely affect the color of the meat with the discoloration predominantly at the edges of the meat. Chicken parts irradiated at -20°F were considered to be more acceptable than those irradiated at 20°F.

Post-Irradiation Studies

Recipe Development

Standard culinary practices can seldom be used for preparing products using irradiated meats because of the requirements for enzyme inactivation and the induced changes in sensory characteristics. Considerable experimentation has been done in the development of recipes, and many acceptable items using irradiated meats as their basic ingredient have been prepared. Research activity in this area is being accelerated as it is now recognized that normal preparation procedures are not always adequate.

Storage Stability Studies

Numerous long term acceptance and storage stability studies have been conducted on radiation sterilized meats. Results of these tests, in which consumer type taste panels periodically evaluated the test items, show that many of the items tested are stable in storage for long periods of time.

The following results have been selected because they show acceptable irradiated products can be produced. Results are not always as favorable as these. The preference scores were obtained by the use of consumer type panels. The products were scored on the nine-point hedonic scale with "dislike extremely" scored as one to "like extremely" scored as nine.

Irradiated bacon, which has now been approved by the U. S. Food and Drug Administration for human consumption, has been well accepted. Table 1 shows that irradiated bacon (4.5 Mrad) will keep without spoilage at non-refrigerated temperatures and will retain good acceptability characteristics for long periods of time even at 100°F.

TABLE 1

Mean Preference Ratings for Irradiated Bacon[a]

Storage Time	70°F Storage	100°F Storage
Initial	7.2	7.0
1 Month	7.1	7.0
4 Months	7.0	7.0
9 Months	7.0	7.0
16 Months	6.9	6.6
25 Months	6.8	6.2

[a]20 Panelists—4.5 Mrad Irradiation.

Irradiated beef steaks have been prepared that received preference scores high in the acceptability range. Table 2 shows the results of a beef steak storage study in which activated charcoal was added to the cans just prior to closure under vacuum. Statistical analysis of the data shows that the benefit of charcoal at the levels used in this experiment were onl, .marginal. Note, however, that all products were consistently scored in the "like moderate" range and remained stable in storage.

Irradiated fresh pork, in general, is fairly well accepted. In most of the studies, the products have been scored in the "like slightly" to "like moderate" range. Results of several studies on the storage stability of irradiated pork products are not too consistent. Some studies indicate that acceptability decreases rather rapidly after about 12 to 15 months at 70°F; whereas, other studies indicate it is stable for two years. Table 3 shows

TABLE 2

Mean Preference Ratings for Irradiated Beef Steaks[a]

Storage Time	Per cent Active Charcoal			
	0.0	0.5	1.0	2.0
Initial	7.1	7.0	6.9	7.0
1 Month	7.1	6.9	7.0	6.7
4 Months	7.0	6.9	7.1	6.9
9 Months	6.6	6.7	7.0	6.8
16 Months	6.8	7.1	6.9	7.0
27 Months	6.4	7.0	6.9	6.9

[a]36 Panelists—70°F Storage—4.5 Mrad Irradiation.

the results of a study on irradiated pork chops in which activated charcoal was added to the cans. Note the high acceptance scores given the product throughout the entire 25-month study, particularly the samples packed with 1 and 2 per cent charcoal.

TABLE 3

Mean Preference Ratings for Irradiated Pork Chops[a]

Storage Time	Per Cent Active Charcoal			
	0.0	0.5	1.0	2.0
Initial	6.5	6.9	6.8	7.2
1 Month	6.7	6.5	7.0	7.4
4 Months	7.0	7.2	7.4	7.1
9 Months	7.1	6.8	7.1	7.1
16 Months	7.4	7.5	7.6	7.1
25 Months	6.7	6.7	7.0	7.1

[a]36 Panelists—70°F Storage—4.5 Mrad Irradiation.

Table 4 shows the results of a storage study on two types of irradiated roast pork loin. The scores in this study indicate the products had fair acceptability when stored at 70°F, but when stored at 100°F, rapid deterioration began after 16 months. The oven cooked product was slightly preferred to the steam co ed product.

As previously mentioned, the use of barbecue sauce tends to mask or react with irradiation flavor. Table 5 shows the results of a study designed, in parts, to determine if the level of sauce had an effect on acceptability. The

TABLE 4

Mean Preference Ratings for Irradiated Pork Loin[a]

Storage Time	Steam Cooked 70°F Storage	Steam Cooked 100°F Storage	Oven Cooked 70°F Storage	Oven Cooked 100°F Storage
Initial	6.4	6.9	6.6	6.8
5 Months	6.2	5.9	6.4	6.0
10 Months	7.4	6.9	7.3	6.7
16 Months	7.0	5.8	7.2	6.1
20 Months	6.4	5.2	6.7	5.2

[a]40 Panelists—4.5 Mrad Irradiation.

TABLE 5

Mean Preference Rating for Irradiated Pork in Barbecue Sauce[a]

Storage Time	Low Level[b] of Sauce 70°F Storage	Low Level[b] of Sauce 100°F Storage	High Level[c] of Sauce 70°F Storage	High Level[c] of Sauce 100°F Storage
Initial	6.4	6.6	7.3	7.0
3 Months	6.6	7.6	7.3	6.6
6 Months	6.4	5.9	6.9	6.5
9 Months	6.9	6.3	6.8	6.1
12 Months	6.5	6.3	6.8	6.0
18 Months	6.5	4.9	6.2	4.7

[a]40 Panelists—4.5 Mrad Irradiation.

[b]22 oz. meat and 6 oz. barbecue sauce.

[c]18 oz. meat and 10 oz. barbecue sauce.

level of sauce did not seem to affect scores, but time and temperature of storage did affect scores. Texture deterioration, which was probably due to the interaction of temperatures and acid in the sauce, seemed to cause the low scores in the 100°F samples that were 18 months old.

Table 6 shows the results of a study on the acceptability of irradiated chicken parts. These preference scores show that chicken can be prepared with good acceptability characteristics and with good storage stability.

Not all irradiated products nor all studies on the products illustrated here have yielded favorable results. Reproducibility continues to be a problem. Whether this is due to lack of control of the raw material, failure to

TABLE 6

Mean Preference Ratings for Irradiated Chicken Parts[a]

Storage Time	Storage Temperature 70°F	100°F	Non-Irradiated Control -20°F
Initial	7.0	7.4	7.2
3 Months	7.4	7.1	7.2
6 Months	7.3	7.0	7.4
9 Months	7.3	7.0	7.4
12 Months	7.1	7.6	7.1
15 Months	6.9	6.8	7.4
18 Months	7.6	7.3	7.3
21 Months	7.4	6.4	6.7

[a]36 Panelists—4.5 Mrad Irradiation.

reproduce processing conditions, or because of difficulties inherent in subjective testing is not known. Studies are now in progress to attempt to determine these factors so we can get reproducibility of results.

Conclusion

This paper briefly summarizes the status of the Product Development Program. It seems clear that processing requirements, starting with the type of raw material and ending with a recipe for preparing the end product, can be established whereby acceptable irradiated meats can be consistently produced.

BIOCHEMICAL ASPECTS OF FOOD RADIATION

A. S. Henick

Introduction

Food is exposed to ionizing radiation solely to prevent microbial spoilage, thereby enabling long storage, in sealed packages, without benefit of refrigeration or need for the older preservation techniques, such as drying, fermentation, or canning. The energy input necessary to inactivate microorganisms is also sufficient to excite reactions in the substrate which may induce changes, desirable, innocuous, or undesirable. To study these changes and to evaluate their impact upon the quality of radiation sterilized foods, an intensive research program is being carried out. The chemistry of radiation-induced change was studied in isolated and purified food components and in selected whole foods. This review will consider the reactions which occur, the products formed, and the over-all effects upon appearance, flavor, odor, and texture of selected food items of military interest.

Food Components

Proteins, lipids, and carbohydrates in varying proportions are major components of most food stuffs. Minor in amount, but often important, are vitamins, pigments, minerals, and flavorants. To a greater or lesser extent, water is also present in most foods. In considering reactions in the wake of ionizing radiation, water is of primary importance. Ions and free radicals produced in water interact with each other and with susceptible molecules in food to account for most of the changes observed. To a lesser extent, principally in dry systems, direct impact of ionized particles with the food may occur. The result in this case is often an activated molecule which may subsequently interact with water if it becomes available.

Environment during and after irradiation affects the reactions which can occur. Although oxidizing ions and radicals are produced in water, oxidations are increased in the presence of molecular oxygen. Effects of temperature are also evident although the data presently available are not sufficient to establish these effects as purely those of temperature on reaction rate, or as changes in the mobility of ions resulting from a change of state in the aqueous phase. In most of the studies reviewed here, irradiations were conducted at temperatures above the melting point of water and in the presence of air.

Carbohydrates

Although carbohydrates are not particularly radiosensitive, the chemical changes which do occur may be important because of flavor or texture changes, especially in foods of plant origin.

Oxidation of hydroxyl appeared to be the principal reaction of monosaccharides in aqueous solution. The primary alcohol at C_6 was oxidized to produce a uronic acid. Mixtures of sugars and amino acids yielded typical browning reaction products. Even greater browning occurred when the sugar and amino acids were irradiated separately in the dry state and then combined as a 50 per cent solution.

Disaccharides were hydrolyzed by very high doses of radiation applied either to crystals or to solutions. Mixtures of sucrose and organic acids gave a browning reaction by some mechanism other than that with hydroxymethylfurfuraldehyde, such as occurs when sugars and organic acids are heated together.

Loss of firmness during the irradiation of fruits and vegetables results from alteration in polysaccharides. Measurable degradation of cellulose occurred not only during but also after irradiation, perhaps by the production of free radicals which decay very slowly. Starches were also degraded. A completely water soluble material was produced from dry starch. In pectins, the principal reaction was the rupture of glycosidic bonds.

Amino Acids

Deamination and carbonyl formation at the alpha-carbon are the principal reactions of amino acids irradiated in aqueous solutions. These and other reactions yielded hydrogen, ammonia, carbon dioxide, formaldehyde, formic acid, the parent fatty acid, the fatty acid with one less carbon atom, the amine with one less carbon atom, and the alpha-keto acid. All reactions occur in both the presence and absence of oxygen, but in oxygen reductive deamination to the acid with one less carbon atom is enhanced.

Deamination of N-terminal amino acids in peptides has been reported. Those within the chain would not be expected to react in this way unless released by hydrolysis. This cleavage has been reported in simple dipeptides, such as glycyl-glycine.

Sulfur amino acids are especially radiosensitive. Cysteine is oxidized to cystine, which undergoes further oxidation to produce hydrogen sulfide and cysteic acid. Methionine may be the precursor of methyl mercaptan found in irradiated meat. When present along with other amino acids in mixtures or in peptide chains, these sulfur compounds exert a sparing effect through preferential reaction.

Aromatic amino acids, phenylalanine, tyrosine, and tryptophane are hydroxylated, and heterocycles in histidine and proline are cleaved.

Some of the amino acid reactions are related to the flavor changes produced by irradiation, which will be discussed later. Although considerable losses of amino acids have been reported resulting from the irradiation of some proteins, no measurable decrease in the content of essential amino acids has been reported in radiation sterilized foods.

Proteins

Denaturation, fragmentation, and polymerization result when proteins are irradiated. Solubility and viscosity of solutions are altered. Changes in solubility continue long after irradiation has ceased. Irradiation of protein in solution leads principally to polymerization, whereas in the dry state, the trend is toward fragmentation.

Binding capacity of protein is altered by irradiation. Binding of anionic dyes is decreased, while that of methionine is increased, thereby providing a sensitive method for detecting molecular changes. Lipoprotein showed less change than did constituent protein. It was also reported that natural lipoprotein protected other protein from radiation-induced changes.

Oxidation has also been reported in irradiated protein, yielding protein peroxides. Oxidative changes in irradiated myoglobin has been related to a pink discoloration which sometimes occurs in meats.

Lipids

Oxidation, decarboxylation, hydrogenation, and dehydrogenation occur when fats and fatty acids are irradiated. These changes, while not affecting the wholesomeness of foods, may have profound effects upon flavor and stability.

Oxidation during and after irradiation is similar to that during thermally catalyzed autooxidation. It depends upon the antioxident content, the number of double bonds, the amount and rate of application of irradiation, the presence of oxygen during and after irradiation, and the time and temperature of post-irradiation storage. In the absence of water and of molecular oxygen, one oxidation occurs for each photon of radiation absorbed, whereas, in the presence of oxygen, typical chain reactions occur. Even saturated fatty acids are oxidized, producing typical oxidation products—peroxides, carbonyls, and reducing compounds. In the presence of oxygen all products are increased, especially peroxides. Antioxidants do not prevent the formation of peroxide in these systems.

Destruction of antioxidants accompanies irradiation in lipid systems. This leads, of course, to decreased stability during subsequent storage.

Minor Components

The fat soluble vitamins are very radiolabile. Vitamin A and carotene are oxidized and the antioxidant activity of Vitamin E (alpha-tocopherol) is destroyed. Water soluble vitamins, ascorbic acid, thiamine, B12, pantothenic acid, and folic acid are rapidly destroyed by irradiation; riboflavin and niacin are relatively stable.

Enzymes, although proteins, are little affected by radiation, at least in the range of sterilizing doses. Although exclusion of oxygen after processing is the equivalent of inactivation of oxidases, heat inactivation is, at present, the sole effective means of inactivation of hydrolytic enzymes, particularly of protease.

Proteases in meat have been isolated and characterized. A number of cathepsin-like proteases have been formed in beef muscle tissue, the principal one of which requires ferrous ions for activity. A number of means for the removal or inactivation of this ion have been investigated for the non-thermal control of protease activity in irradiated meat. Best results have been obtained through alteration of the pH in the tissue, to below pH 5 or to exactly pH 7. Alteration of ionic strength, as with sodium chloride, also appears helpful. Techniques for accomplishing these alterations, such as perfusion, stitch pumping with buffers, and treatment with ion-exchange resins are currently under study.

Flavor Changes

Flavor changes accompany nearly all food processing. In fact, some processes are used primarily to achieve desirable flavor changes. Since flavor response is subjective, the desirability of any given flavor change depends, in the last analysis, on the consumer. Most testers have agreed that some of the flavor changes produced in radiation sterilized meat are undesirable. This alone would be sufficient justification for the extensive research which has been undertaken to identify, and thereby to control, the flavor changes accompanying radiation in meat.

Volatiles

In an elegant study, still continuing, slurried beef was irradiated while volatile substances were distilled off. The collected volatiles were carefully fractionated by vapor chromatographic procedures and the chromatographically pure fractions identified. Comparisons were made with volatiles of unirradiated beef and with those collected from beef irradiated prior to distillation. To date 41 compounds have been positively identified. As shown in the tables, there are hydrocarbons (Table 1), alcohols (Table 2), aldehydes (Table 3), and ketones (Table 4). Not shown are the extremely volatile substances such as ammonia, hydrogen sulfide, etc. which are also present. It can be seen

TABLE 1

Volatiles in Beef - 1

Compounds	Irradiated			Not Irradiated	
	Concurrent	Not Concurrent		Concurrent	Not Concurrent
		Fresh	Stored		
Hydrocarbons					
N - Decane		?			
1 - Decene		?			
N - Undecane		X	X		
1 - Undecene		X	X		
N - Dodecane		?	X		
1 - Dodecene		X			
N - Tridecane	X		X		
1 - Tridecene	X		X		
N - Tetradecane			X		
Benzene					X

TABLE 2

Volatiles in Beef - 2

Compounds	Irradiated			Not Irradiated	
	Concurrent	Not Concurrent		Concurrent	Not Concurrent
		Fresh	Stored		
Alcohols					
Ethanol	X	X	X	X	X
Iso-Propanol	?	X		?	X
N - Butanol	X	X	X	X	X
Iso-Butanol	X		X		
2 - Butanol		X	X		
N - Pentanol	X	X	X	X	X
Iso-Pentanol	X		X		
N - Hexanol	X		X		
N - Heptanol	X	X	X	X	X
N - Octanol	X		X		?

TABLE 3

Volatiles in Beef - 3

Compounds	Irradiated			Not Irradiated	
	Concurrent	Not Concurrent		Concurrent	Not Concurrent
		Fresh	Stored		
Aldehydes					
N - Butanal	X		X		
N - Pentanal			?		?
Iso-Pentanal	X	X		?	?
N - Hexanal	X	X	X	X	X
N - Heptanal	X	X	X		X
N - Octanal	X	X	X		X
2 - Octanal			?		
N - Nonal	X	X	X		X
N - Decanal	?		?		
N - Undecanal	?	?	X	?	?
Benzaldehyde	X	X	X		?
Phenylacetaldehyde	X				
Methional	X		X		

TABLE 4

Volatiles in Beef - 4

Compounds	Irradiated			Not Irradiated	
	Concurrent	Not Concurrent		Concurrent	Not Concurrent
		Fresh	Stored		
Ketones					
Acetone			X		
2 - Butanone	X	X	X		X
Acetoin	X	X	X	X	X
Diacetyl	X				
2 - Nonanone			X		
2 - Undecanone	X	X	X	?	?
Misc.					
Ethyl Acetate	X				
Dimethyl Sulfide					?
Unknowns	18	21	18	11	13

that aliphatic hydrocarbons are products of irradiation, that alcohols, aldehydes, and ketones are found in both irradiated and unirradiated volatiles. The amounts of these compounds from irradiated beef is, however, much greater than from the unirradiated. Nitrogenous bases were not expected since at the pH of meat they were not volatile.

The relationship of some of these identified substances to the off-flavor formed in beef by radiation sterilization was studied using a trained sensory panel. Employing an index of similarity technique, proper dilutions were determined for 29 of the then identified chemicals in irradiated beef volatiles. These were then presented to the panel on raw ground beef, as carrier, for comparison with irradiated ground beef. Table 5 shows the 11 compounds which exhibited the highest degree of similarity to the odor of irradiated beef.

In the second step of this study these 11 compounds were paired for testing. Thirteen pairs, as shown in Table 6, were reported to have scores of four or higher in radiation intensity, compared to the irradiated beef standard which had a score of six. A number of blends containing three or four of the selected compounds were also tested. Of these, four were reported to have scores of four or higher. These are shown in Table 7. It can be seen that

TABLE 5

Radiation Odor in Beef - 1 (Single Chemicals)

1.	Pyridine
2.	Hydrogen Sulfide
3.	Propanal
4.	Methylamine
5.	Acrolein
6.	Methional
7.	2 - Butenal
8.	Propyl Mercaptan
9.	Carbon Disulfide
10.	Dimethyl Sulfide
11.	Acetaldehyde

TABLE 6

Radiation Odor in Beef - 2 (Paired Chemicals)

1.	Dimethyl Sulfide, Propanal
2.	Hydrogen Sulfide, Methalamine
3.	Propyl Mercaptan, Dimethyl Sulfide
4.	Dimethyl Sulfide, 2 - Butenal
5.	Dimethyl Sulfide, Methylamine
6.	Propyl Mercaptan, Methylamine
7.	Propyl Mercaptan, Pyridine
8.	Carbon Disulfide, Acrolein
9.	Hydrogen Sulfide, Pyridine
10.	Acetaldehyde, Methylamine
11.	Dimethyl Sulfide, Pyridine
12.	Carbon Disulfide, Acetaldehyde
13.	Carbon Disulfide, Pyridine

TABLE 7

Radiation Odor in Beef - 3 (Chemical Blends)

1.	Methylamine, 2 - Butenal, Dimethyl Sulfide
2.	Propyl Mercaptan, Acetaldehyde, Dimethyl Sulfide
3.	Propyl Mercaptan, Acetaldehyde, Methylamine, Octane
4.	Dimethyl Sulfide, Pyridine, Acrolein

each blend contains a sulfur compound, a carbonyl compound, and a nitrogen base. It is also apparent that, except for blend three which contains a hydrocarbon, all of these blends contain compounds which have been found in the volatiles of unirradiated beef, although not necessarily in the studies reviewed here. This finding lends support to the contention that radiation odor does not result from products unique to radiation, but rather from changes in the amount and proportions of odorous materials commonly found or produced in meats by other energy means.

Proteins

Two studies have been conducted in order to implicate specific protein precursors in the formation of the unique radiation flavor in beef. In one study, a low molecular weight, water soluble protein fraction of beef was formed to exhibit the odor when irradiated in aqueous solution, but not in the dry state. Upon wetting with water, the dry irradiated protein fraction exhibited the odor. The odor was quenched by treatment with reagent specific for sulfhydryl groups and recurred when the effects of these reagents were nullified.

In the second study, a small compound which separated from an extract of phospholipids from beef tissue was found to exhibit the radiation odor strongly. Although not yet completely characterized, it appears that this compound may be a glycoprotein and that it may be the same compound as that in the first study.

In yet another study, an attempt was made to relate the off-odor in irradiated beef to free radical reactions in the proteins. The free radicals were measured by electron spin and were found to be very stable in frozen or dehydrated systems. Upon thawing of the frozen system or addition of water to the dehydrated system, rapid decay of the free radicals was accompanied by the release of odor. Attempts to characterize the free radicals present, their decay products, and the composition of the odor volatiles were not successful. It was noted, however, that the protein fraction used in the earlier reported studies was an ideal substrate for this study.

Texture

Softening of meat was reported early as an effect of radiation sterilization. Apart from the usefulness of this observation in permitting use of the less tender cuts and grades of carcass in military feeding, it was interesting to study the mechanism of this softening. In a careful study involving several cuts in different grades of beef, it was reported that the principal change associated with tenderization in irradiated beef was degradation of collagen. The degradation was chemically similar to but much more rapid than that in meat held at moderate temperatures, as in a smoke house.

Another possible cause of texture change in radiation sterilized beef is, of course, autolytic enzyme activity since the enzymes are not inactivated by the sterilization dose. In practice this problem is avoided by a heat treatment to inactivate the enzymes prior to sterilization. In addition to the chemical treatment referred to above, other techniques for non-thermal enzyme inactivation are being considered. One, which appears to have a high probability of success, involves selective application of radio-frequency energy in a manner to preclude temperature change.

Future Plans

During the next two years it is expected that the studies on odor volatiles, on chemical inactivation of enzymes, and on the apparent precursor of radiation odor will be completed. The feasibility of employing radio-frequency energies to inactivate enzymes without heat should be determined and a program develped for exploiting the method in processing.

New developments in processing technology, particularly in the relationship of processing temperature to sterilization, and the probability of reduction in safe sterilizing dose resulting from advances in microbiological studies will require re-evaluation of chemical changes. The effects of temperature, dose rate and total dose on the chemical changes involving flavor, texture, and storage stability remain to be established.

New and improved techniques in the measurement of the parameters of texture, being developed in other programs of Food Division, will be employed in extending knowledge of texture and textural changes in irradiated meats and in establishing means for quality control.

In support of future petitions to FDA for the clearance of radiation sterilized meat items, careful studies are in progress to determine amounts and rates of accumulation of deterioration products during irradiation and post-irradiation storage. The data developed in these studies will be useful in quality control and storage stability prediction, not only in the Radiation Program but also in the other Programs of Food Division.

RADIATION MICROBIOLOGY
PROGRAM PHILOSOPHY, PRINCIPLES, AND OBJECTIVES

Hamed M. El-Bisi

It is a privilege to have this opportunity to appear before this audience and comment briefly on this very stimulating and very challenging aspect of the Radiation Research and Development Program.

I consider it important to reflect a few personal thoughts on this subject in view of the current change in organization and personnel. All of the microbiological activities within the Food Division are now consolidated and coordinated in the newly developed Microbiology Branch. Radiation microbiology occupies the foreground of our expanded program in the areas of production, preventive, and control microbiology.

Prior to setting the broad perspective for our program in radiation microbiology, let us examine very briefly the major events in the history of thermocanning, as well as the principle underlying its preservative action. It is from this long established wealth of know-how and industrial experience that we can derive the basic elements to our foundation as well as the basic directives to our future plans.

It has taken over a century to develop a scientific quantitative basis for the thermoprocess. In 1809 Nicholas Appert, the French confectioner-baker was awarded 12,000 francs by the Napoleonic Government for his introduction of the first successful thermocanning process. It might be interesting to note that the motive was triggered by the logistic needs of the French Army. Half a century later, in 1860, Pasteur was the first to recognize the principle underlying the preservative action rendered by Appert's empirical process. Pasteur explained that the heat destroyed the microbial life, and the hermetic seal prevented re-entry of new contaminants. Although it was indeed a revolution in food economics and technology, the public had to bear undue economical as well as human losses as a result of the empirical approach to thermocanning. It was not until 1908, a century later, that Chick introduced the "first order" as that of death of microorganisms. It was not until the late 1910's and through 1920's that food microbiologists such as Bigelow, Esty, Williams, Meyer, Underwood, Prescott, and Ball developed the first sound scientific quantitative approach to thermocanning. Their efforts led to the introduction of several mathematical, graphical, and nomogramical methods that we now use widely and reliably for the computation of a thermoprocess.

The principle underlying the preservative action of thermocanning is still essentially as Pasteur introduced it; namely, the destruction of practically all pathogenic and most deteriorative microorganisms by heat, and the prevention of post-process contamination by hermetically sealing the container.

The principle underlying the theoretical computation of a thermoprocess is the ability to integrate the microbiocidal action of heat being transferred through a given product during a given process. The integration process requires the establishment of two types of data: (a) physiological, i.e., the kinetics of thermal destruction of an index microbial population, and (b) physical, i.e., the kinetics of heat transfer through the product under processing conditions.

The kinetics of thermal destruction are generally expressed in terms of two primary rate constants: the D value ($2.3k^{-1}$), a death rate constant which expresses the time for 90 per cent destruction at a given temperature; and z value ($18/\log Q_{10}$), an empirical temperature coefficient which describes the temperature (°F) difference for 10X change in the D value.

Both the D_{250F} and z values for Clostridium botulinum spores were selected as the reference parameters for establishment of the thermal dose requirement; these spores being the most thermoresistant pathogen known. To provide maximum safety, a lethality equivalence of 12 x D_T has been long regarded as the minimal thermal dose requirements; hence the birth of the so-called 12-D concept.

However, it was soon realized that other mesophilic saprophytic spoilage microorganisms are of higher degree of thermoresistance than C. botulinum. C. sporogenes (NCA PA #3679) has been regarded as the mesophile of the highest order of thermoresistance; and, hence, is conventionally used as the index for establishing a thermoprocess. A 5 D_T lethality equivalence has been recommended as the minimal thermal dose requirement, a value which normally supercedes the 12 D_T for C. botulinum and hence provides an added measure of public health safety.

The principles for thermocanning have prevailed for over forty years, throughout which no single case of botulism intoxication (as a result of the inadequacy of the prescribed thermoprocess) has been reported.

An exception to the above rules is exemplified in the case of thermocanned cured meats (the nonperishable shelf type). Here, the product receives a much reduced thermal treatment, normally of a lethality equivalence less than one-tenth the minimum prescribed for low acid products. In this case, however, the process variables provide a unique environmental circumstance, shown by over fifty years of industrial practice to uphold the microbiological safety and stability of the product. If such circumstance fails, it is claimed that one would usually encounter a rapid saprophytic case of spoilage, which is readily detectable and therefore serves as a built-in warning system. However, serious research effort is still needed to define the "unique circumstances."

Only recently (late 1940's) [illegible] microbiologists triggered vigorous interest in studying the physiological mysteries of the spore cell. Significant scientific reports have accumulated over the past fifteen years dealing with the cytological, biochemical, and biophysical aspects of the spore cell. Through such efforts, our ultimate hope is to define the key cellular factors responsible for the unique resistance of the spore cell to varied adverse energies. Breakthroughs along this path of basic knowledge may lead to a revolution in thermoprocessing principles.

It is in the light of this brief analytical review of a long and well established know-how and industrial experience that we project and derive the philosophy and principles of our Radiation Microbiology Program.

The potential of electromagnetic radiation as an effective means for the destruction of biological forms has been recognized since the introduction of X-rays by Roentgen in 1895. The recognition of ionizing radiation as a potential means for microbial destruction and consequently a potential means for radiocanning is recent history, born by the contemporary advance in man's effort to harness the atomic energy for peaceful purposes. The diversified and far-reaching research and development program led by the U. S. Armed Forces Food & Container Institute (presently, the Food and Container Divisions, U. S. Army Natick Laboratories), in cooperation with numerous academic, industrial, and other governmental agencies for over a decade, has laid the cornerstone in the science and technology of Radiation Preservation of Foods.

Radiocanning is synonymous with thermocanning in principle and objective. There are, however, basic procedural differences between the two preservation methods:

First and by virtue of nomenclature, there is the difference in the main destructive energy utilized.

Second, there is the difference in the laboratory "index of resistance." Whereas thermomicrobiologists have been spared the nuisance and hazard of having to adhere to Clostridium botulinum spores as a thermoresistance index, radiomicrobiologists must use certain BOT types, being the most radioresistant spore populations yet to be reported, as radioresistant indices.

Third, there is evidence that BOT toxins, other asporogenous bacteria, and food enzymes, which are naturally thermolabile, are far more radioresistant than BOT spores. Therefore a heat treatment to destroy asporogenous forms and to inactivate toxins and enzymes became an essential adjunct to radioprocessing, leaving BOT spores as the essential index of radioresistance and consequently the determinant of the minimal radiation dose requirement.

Fourth, the physiological contrast between a thermo-killed and a radio-killed BOT spore constitutes another potential difference. Whereas lethal thermal energy concurrently inactivates practically all of the spore's endogenous physiological constituents and/or activities, experimental evidence

indicates that lethal radiation energy leaves potentially significant residue. Such post-mortem residue implicates BOT toxin as either an inherent spore constituent or as a product of post-mortem anabolic activity.

A fifth contrasting feature which requires deeper and more serious consideration is the 12-D concept of safety, D here being the radiation dose necessary for 90 per cent reduction of the index spore population in the prototype product. It has been proposed and generally accepted that, based on a general experimental estimate of a BOT spores' D value of about 0.375 Mrad, the minimal radiation dose should become 4.5 Mrad.

While this concept serves as an adequate conservative starting point, it must be subjected to more elaborate and careful assessment for its experimental validity, philosophy, and process applicability.

Experimentally, full recognition and evaluation must be given to a multitude of factors which have long been recognized to influence a D value. A general indiscriminate use of widely heterogeneous collection of data for the rough calculation of a single D value, to be applied indiscriminately for all products and all processes, is unscientific. Furthermore, the insistence upon demonstrating an actual 12-D reduction in an inoculated-pack's spore population is scientifically unsound, and, in my opinion, an outdated and unwarranted experimental venture.

In order to determine the minimal radiation dose for a given prototype product, one may carry out the following procedural steps, executed within a sound experimental design, and in the following order:

1. Establish cultural, preparatory, and handling procedures for the provision of large amounts of stable, highly radioresistant, and reproducible stock spore suspensions of representative test strains.

2. Establish the comparative radioresistance pattern of test strains in the prototype substrate in a laboratory system (e.g., TDT cans or tubes).

3. Select the highest radioresistant strain. Establish a statistically reliable estimate of the D value in the prototype substrate, in a laboratory system, taking into account the following basic factors:

   a. Spoilage data (in normal and enriched prototype substrate) versus post-radiation survival data.

   b. Effect of variation in the initial inoculum level over an appropriate range.

   c. Appropriate statistical treatment of data to arrive at the most reliable and safe estimate of D.

4. Design and carry out an appropriate prototype inoculated pack test. The experimental design is in no way intended to demonstrate an

exaggerated safety sterility level, but primarily to ascertain various computed reduction levels, based on both survival and partial spoilage data.

5. Based on the above effort, one can safely and reliably report:

   a. A reliable D estimate for the product in question.

   b. A minimum sterilization dose level (analogous to the TDT constant F_O) based on the extrapolation of the final D estimate to any level deemed appropriate.

Furthermore, with regard to the philosophy behind the 12-D concept, one is still tempted to add the following thoughts:

1. The adherence to the concept by the thermocanning industry is simply due to its process feasibility and technological necessity.

2. The concept is frequently by-passed in cases where technological necessity arises and the processor still often relies upon warehouse and accelerated stability tests.

3. It would be more effective to establish vigorous sanitary and pre-processing microbiological quality control thereby allowing realistic minimal radiation doses, than to unduly exaggerate the requirement to the point of impracticability.

4. More serious attention should be given to:

   a. The actual incidence of botulism in raw products in question.

   b. An assessment of actual industrial practices with regard to the thermoprocess levels.

On the other hand, a word of caution is necessary for those who contrast the premises of thermoprocessing with those of radioprocessing in the case of cured meat and perhaps other "environmentally armored" products. At sub-lethal energies, one would anticipate a difference in residual ecology as well as physiological activity of surviving spores. The pattern of resistance to heat is different from that to radiation, hence a residual built-in saprophytic spoilage indicator in the latter case is questionable. The effect of sublethal levels of radiation upon the physiological capabilities of residual viable BOT spores is also a significant question.

The approach to the feasibility question, however, will remain largely dependent on the furthering of our basic knowledge of the effects of the multitude of cellular, environmental, and process variables upon radioresistance. Preliminary findings along these lines are promising.

In summary, may I bring into focus the main objectives underlying the present Radiation Microbiology Program and state the present broad lines of research and development activities.

Objectives

1. Provide quantitative radiation destruction data essential towards the establishment of the microbiological safety and stability of prototype irradiated food products.

2. Seek fundamental knowledge of the cellular, environmental, and process factors which influence or govern radioresistance, in an attempt to broaden the spectrum of applicability of radiocanning and to further improve the quality and economics of earlier prototypes.

Current and Future Research Activities

1. Continue studies on the comparative radioresistance of Clostridium botulinum spores in selected prototype ration substrates. This phase was completed for bacon, is being completed for chicken parts, and will be initiated for pork followed by ham and beef. It will be extended to cover other bacterial types in an attempt to provide a nontoxigenic index of radioresistance.

2. Continue studies to establish the microbiologically safe radiation dose for prototype ration products. Data from these studies will be used to support FDA petitions for the clearance of said products for unrestricted human consumption. This phase has been completed for bacon. It will be initiated for the chicken pack and later for pork, ham, and beef.

3. Continue studies to reduce the minimal radiation dose requirement via biochemical and chemical additives. Initially, beef will be used as the test substrate because of its greater susceptibility to adverse effects of radiation on organoleptic properties. Prototypes, other than beef, may be included such as chicken, ham, and/or pork.

4. Continue studies on effect of unit-process variables on bacterial radioresistance in an attempt to further reduce the minimal radiation dose requirement. Effect of low temperature shock (-20° to $10^{\circ}C$), both pre- and post-irradiation, upon spore radioresistance will continue in model systems, followed by prototype ration substrates, in which beef will be the priority item.

5. Combined sporicidal effects of varied elevated temperatures and irradiation levels, applied simultaneously and/or sequentially, will be evaluated in model systems, followed by prototype ration substrates. Preliminary feasibility tests will determine the prototypes to be selected.

6. Continue studies on effect of unit-process variables on radioresistance in an attempt to differentially enhance product stability and still effect

microbiological sterility. Studies on spore radioresistance at very low temperatures in the range of -196^{o} to $-20^{o}C$ will be continued with the emphasis on D value and end-point destruction determinations. Studies will be conducted in model systems, followed by prototype ration substrates; here again beef will be given priority.

7. Combine radiation at very low temperatures with other biochemical, chemical, and biophysical factors.

8. Initiate studies on the mechanism of cellular radioresistance in an attempt to recognize some of the basic cellular factors responsible for its death by and/or resistance to radiation.

9. Initiate studies on the radiostability of botulism toxins and their inherent physiological properties with reference to irradiated foods. The initial stage will ascertain the kinetics of toxin inactivation by ionizing radiation in model systems and prototype ration substrates.

10. Continue comparative studies of the sporicidal as well as the preservative action of both thermal and radiation processing of cured meats in an attempt to establish equivalence levels of safety.

11. Continue studies on the incidence of C. botulinum and anaerobic spore populations in raw meats.

12. Initiate studies on the incidence and radioresistance of viruses and rickettsiae in irradiated foods.

Finally, I wish to acknowledge with pride and sincerity the profound and accreditable task accomplished by the principal investigators who led this program during the past three years: Dr. Nicholas Grecz, Mr. Abe Anellis, and their co-workers. Their dedication and persistence have started the program in an excellent manner.

RADIATION MICROBIOLOGY PROGRAM PROGRESS

Nicholas Grecz, Abe Anellis, and Hamed M. El-Bisi

Introduction

The period 1961-1963 was characterized by a marked increase in the radiation microbiology program of the Army. The program has covered three major areas of activity.

1. Fundamental aspects: the effect of cellular, environmental, and process factors on spore radioresistance.

2. Applied aspects: prototype product studies to determine their minimal radiation dose requirement.

3. Microbiological control activities: the monitoring of microbiological quality and toxigenicity of prototype foods under development.

Comparative Radioresistance of C. botulinum

In the early 1950's it was first realized that the radioresistance of spores of *C. botulinum* was equal to or greater than the radioresistance of spores of representative non-toxigenic food spoilage organisms.[1,2] This observation focused attention on the necessity of further study of the resistance of spores of *C. botulinum* to ionizing radiation. Subsequent research by Kempe *et al* (1954),[3] Kempe (1955),[4] Wagenaar and Dack (1956),[5] Denny *et al* (1959),[6] and Pratt *et al* (1959)[7] have confirmed the exceptionally high radioresistance of *C. botulinum*.

Recent studies of Anellis and Koch[8] revealed a wide variation in radioresistance of individual types and strains of *C. botulinum*. A total of 102 strains of *C. botulinum* (56 strains of type A, 43 type B, and 3 non-toxigenic strains which could not be typed) were examined by Anellis and Koch for resistance to gamma irradiation. When test organisms were suspended in neutral phosphate buffer at concentrations of 10^4 spores per tube, the threshold sterilizing dose appeared to be 1.4 Mrad. Partial survival at 1.4 Mrad was shown by 10.7 per cent of the type A strains, 18.6 per cent of the type B strains, and one of the three non-toxigenic strains. In general, type A strains indicated higher radioresistance than type B strains, although there was overlapping. Representatives of the most resistant strains had D values of 0.317 to 0.336 Mrad; the D values of an intermediate group were 0.224 to 0.253

Mrad; the most sensitive strain studied, 51B, had a D value of 0.129 Mrad. The radioresistance of Putrefactive Anaerobe 3679, strain S-2, was (D = 0.209 Mrad) comparable to the intermediate C. botulinum group (Table 1 and Fig. 1).

TABLE 1

Comparative Radioresistance of Representative Strains of Clostridium botulinum types A, B, and PA 3679

Type	Strain No.	Mean D value
		Mrad
A	33	0.334
A	36	0.336
B	40	0.317
B	41	0.318
B	53	0.329
A	62	0.224
A	77	0.253
A	12885[a]	0.241
B	9	0.227
B	51	0.129
PA	3679 S-2	0.209

[a]Toxigenic variant.

Factors Affecting the Radiation Resistance of C. botulinum

Suspending Menstruum

Inhouse studies established that food substrates such as ground meat or broth exert a greater or lesser protection against the lethal effect of ionizing radiation on spores of C. botulinum. The protective effect of food substrates as compared with buffer appears to depend on the temperature during irradiation. The changes in radiation resistance of spores in various suspending substrates were particularly pronounced at irradiation temperatures ranging from approximately -10° to 70°C. This range includes the so-called "ambient" temperatures which are of practical significance for radiation sterilization applications.[9]

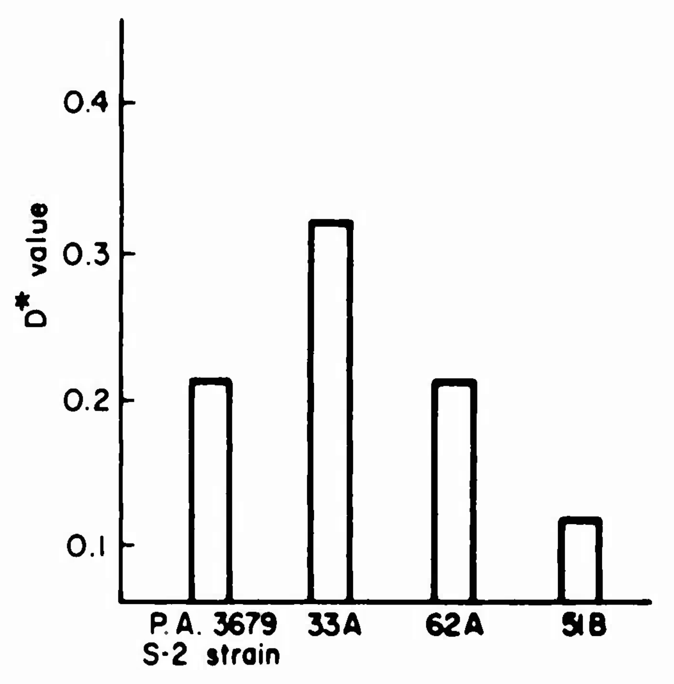

Figure 1. Comparative radioresistance of PA 3679 and representative strains of Clostridium botulinum; 10^4 spores per tube in phosphate buffer, pH 7.0.

Survivors of C. botulinum 33A after 0.7 and 0.9 Mrad of gamma radiation from Co^{60} were determined in beef dinner, borate buffer, and tris buffer (all at pH 6). The temperature during irradiation was controlled at -196°, -150°, -100°, -50°, -20°, 0°, 20°, 40°, 60°, 80° and 90°C. The temperature-survival curves formed a plateau which was unaffected by temperature variations at the region below -50°C (beef dinner) and -100°C (borate and tris buffers) at both radiation levels, 0.7 and 0.9 Mrad. The lowest radiation resistance of spores represented a shallow trough in the temperature-survival curves, with minima at the following temperatures:

	0.7 Mrad	0.9 Mrad
Beef dinner	0°C	40°C
Borate buffer	-20°C	0°C
Tris buffer	20°C	-

Following said temperature ranges, the radiation resistance of spores steadily increased to form a sharp peak which, again, varied according to substrate and radiation dose received, and was as follows:

	0.7 Mrad	0.9 Mrad
Beef dinner	90°C	85°C
Borate buffer	75°C	65°C
Tris buffer	65°C	-

The temperature-survival curves obtained in the three suspending substrates did not intersect, except for the portion of the curves which is known as the "paradoxical inversion of the radiation sensitivity of spores;" in the latter portion there was a considerable amount of overlapping of all three curves.

pH

Several studies including some recent inhouse work indicate that pH does not noticeably affect radiation resistance of spores of C. botulinum as long as the temperature during irradiation is kept at "ambient" or lower levels. However, at temperatures 50°C and above, the pH has a very pronounced effect on radiation resistance of spores of C. botulinum. The spores appeared to be most sensitive in the acid region and least sensitive in the alkaline region. Curiously, at a narrow pH band around pH 6 the spores appeared to be exceptionally sensitive to radiation at ambient and elevated temperatures; the dip in survival curve at pH 6 was most pronounced at elevated temperatures.[10]

Chemicals

Several chemical compounds are known to exert a synergistic effect towards the X-rays inactivation of aerobic spores of Bacillus coagulans (thermoacidurans), B. subtillis, and B. cereus. Among these were ethylene oxide, propylene oxide, and sorbic acid. Recent work at Oregon State

University revealed that iodoacetic acid in minute quantities significantly reduced the radiation resistance of C. botulinum and Micrococcus radiodurans.

A survey of a large number of conventional food additives in lean ground beef (Oregon State University) indicated that mustard (allyl-isothiocyanate), sodium chloride, nitrites, and nitrates reduced the radioresistance of C. botulinum strains 5A and 155B. However, more recent results indicate that the radiosynergistic activity of nitrites and nitrates was not as pronounced with strain 33A. Inhouse experiments indicated that monosodium glutamate in ground beef had very little or no effect upon the radioresistance of C. botulinum.

Temperature

Early work of Kempe et al[4, 11, 12] demonstrated that a relatively low dose (one Mrad) sensitized spores of C. botulinum or PA 3679 to subsequent heating. However, heating prior to irradiation did not sensitize spores to the lethal effect of radiation. In an additional series of experiments, heat and radiation were applied simultaneously. When the number of surviving spores was taken as an index of radiation resistance, the experiments appeared to indicate that simultaneous application of heat and radiation resulted in an increased survival of spores. However, recently completed redesigned experiments in our laboratory suggest that, on the basis of partial spoilage data, end-point destruction of spores of C. botulinum is an almost linear function of temperature. The spores were most radiation sensitive at higher temperatures (90°C) and least sensitive at low temperatures (-196°C). Apparently, the two different experimental designs in this case lead to diametrically opposite conclusions as to the radiation resistance of spores of C. botulinum at elevated temperatures.

From a series of three inoculated pack experiments with spores of C. botulinum 33A in ground beef, a general conclusion can be made that low temperatures offer a significant degree of protection to spores of C. botulinum. The protective effect of low temperatures appears to depend on the size of the spore inoculum and on the composition of the suspending medium. These experiments were carried out in an apparatus consisting of a Dewar flask and a relay system controlling the flow of liquid nitrogen. This apparatus permitted the irradiation of samples in tin cans or pyrex tubes at any temperature ranging from 0° to -196°C with an accuracy of ±1.5°C.

An inoculated pack comprising 320 cans of ground beef containing 5×10^4 spores of C. botulinum 33A per can was irradiated with gamma rays from Co^{60} at -196° and 0°C. Incubation was carried out at 30°C for 6 months. Approximately an extra 0.9 Mrad radiation dose was required to inactivate the spores at -196°C than at 0°C. Samples treated at -196°C showed partial spoilage at 3.6 Mrad and no spoilage at 3.9 Mrad; the corresponding doses at 0°C were 2.7 and 3.0 Mrad respectively. The majority of positive cans swelled in 2 to 14 days with occasional swelling occuring as late as 33 days. At 1.0-1.5 Mrad,

swelling of cans was more rapid than in unirradiated controls; whereas at progressively higher doses swelling was delayed proportionally to the radiation dose received. The remaining non-swollen cans had no toxin after six months ot storage, although occasional cans contained dormant spores.

The radiation resistance of C. botulinum 33A in phosphate buffer also increased at -196°C as compared with 0°C. The D value was 0.462 Mrad at -196°C and 0.275 Mrad at 0°C.[13]

A second low temperature inoculated pack experiment comprising 490 irradiated cans of ground beef was designed to test the resistance of spores of C. botulinum 33A as a function of temperature in the range from 25° to -196°C (at 25°C intervals). Swelling of the cans during six months of incubation at 30°C indicated that the radiation sensitivity of spores gradually decreased as the temperature of irradiation was lowered. There appeared to be no sudden breaks in the radiation sensitivity curve of strain 33A over the range between 25° and -196°C.[10]

A third low temperature experiment comprising over 1200 cans of ground beef was designed to test the effect of spore load on the radiation sterilization dose in the temperature range between 95° and -196° C (at 25°C intervals). Three spore inoculum levels (10^3, 10^5, and 10^7 spores per can) of the highly radiation resistant strain 33A of C. botulinum were used in this experiment. The cans were incubated at 30°C for 8 months and subsequently evaluated for swelling, toxin, and any viable organisms remaining in the beef but not causing swelling. The results indicate that the difference in radiation resistance between the 10^7 and 10^3 spore load was relatively large at -196°C.

Microwaves

An inhouse study on the basic mechanism of action of radiofrequency energy in the microwave region (2450 mc) on spores of PA 3679 indicates that microwaves were consistently more lethal to spores than conventional heat. However, the difference between the two energies was not very large. The data indicate that the largest difference between the lethal effect of the two energies was at 85°C. This difference decreased gradually as the temperature during irradiation was raised and eventually became nil at 95°C.

Properties of C. botulinum Spores Related to Their Radiation Resistance

Toxicity

Residual toxicity in meat products from spores of C. botulinum was first observed by Kempe and Graikoski.[14] Subsequently, it was pointed out by Grecz, Anellis, and Schneider[15] that C. botulinum types E and B spores produced no heat resistant toxin; whereas heat-shocked type A produced approximately 2 to 3 x 10^7 MLD (mouse lethal doses) per spore. The toxicity of

heat-shocked spores appears to be associated with the spore itself and not with vegetative growth or enzymatic synthesis of new toxin within the mouse.[16]

In our own experimental practice inocula of 10^6 - 10^7 botulinum spores, i.e., an equivalent of 1-10 MLD of heat-stable botulinum toxin, will require 1.5 to 3.0 Mrad for complete kill of the spore population. The radiation dose of 1.5 to 3.0 Mrad has probably no significant effect on the potency of the toxin within the spore since preformed botulinum toxin in food is known to be resistant to as much as 7 Mrad.[5]

Tailing

Radiation survival curves determined by Wheaton and Pratt[17] for *Clostridium botulinum* consisted of two distinct portions: at low radiation doses (up to approximately 2.0 Mrad) the curves declined exponentially compatible with the classical single hit theory; whereas at higher radiation doses (2 to 5 Mrad) the curves tended frequently to level off forming the so-called "tail." The "tail" portion persisted regardless of the suspending medium or the initial spore concentration.

Similar "tailing" of radiation survivors was observed by Brown *et al*[18] with PA 3679 in cured ham or raw pork and by Gunter and Kohn[19] and Koh *et al*[20] with non-sporeforming bacteria.

Factors affecting the survival of occasional spores of *C. botulinum* 33A at radiation doses beyond the exponential range of the survival curves, i.e., the "tail" portion, have been investigated by Anellis, Grecz, and Berkowitz (manuscript in preparation). Survival of spores was observed up to 9.0 Mrad with erratic skips at doses between 2 and 9 Mrad. Spore crops produced from 9.0 Mrad survivors had no unusual radiation resistance as compared with parent spore crops. The resistance of spores was significantly higher in pork-pea broth than in phosphate buffer pH 7. The materials released into these two suspending media from spores exposed to 4.5 Mrad appeared not to be responsible for increased resistance such as manifested by "tail" survivors. Furthermore, dense populations of spores inactivated by 4.5 Mrad did not offer any detectable degree of radiation protection to viable spores of *C. botulinum* 33A.

Physiology and Cytology

Studies on the influence of ionizing radiation on the physiology of spores of *C. botulinum* were carried out in an effort to elucidate the mode of action of ionizing radiations and to gain control over its lethal effect on spores. Spores were fractionated into major structures, and an investigation of the enzyme make-up and the morphology of irradiated and non-irradiated spore material undertaken. The results of recent experiments indicate that irradiation up to 9 Mrad does not affect spore refractility or impermeation to simple strains,

but causes the loss of all dipicolinic acid (DPA). Three Mrad caused the loss of approximately 10 per cent DPA of C. botulinum 62A spores. The percentages of material released upon irradiation, on a dry weight basis were:

	Total	DPA
Unirradiated controls	1.6	0.1
3 Mrads	4.9	1.0
6 Mrads	21.0	ca. 10
9 Mrads	25.5	ca. 11

The amount of ninhydrin positive material liberated showed a sharp increase with increasing radiation dosage at levels above 3 Mrads. Part of this material was non-dialyzable protein, i.e., of a large molecular size.

From these studies it is apparent that under certain conditions the common properties of viability, impermeation to simple staining, phase refractility, and exudation are affected singly. These findings appear to be contrary to the concept of interdependence of these properties. The study also revealed that the characteristic refractility of spores was somehow linked to insoluble phosphates of several metal ions, whereas DPA was not essential for spore refractility. Thus, 85 per cent of the spores which lost all DPA as the result of irradiation to 9 Mrad still remained refractile. On the other hand, insoluble phosphates of several metal ions restored refractility of spores which were made non-refractile by germination in yeast extract, or by heating. Dipicolinic acid, which is present in all spores and absent from vegetative cells, seems to be located outside the spore permeability barrier.

Prototype Product Studies

The establishment of an adequate radiation sterilization dose for varied prototype food products is the prime objective of the research activities of the radiation microbiology group. This effort consists primarily of experiments in which prototype food packs are inoculated with spores of C. botulinum and irradiated under simulated process conditions.

The experimental pattern consists of three major steps:

1. Selection of the most radioresistant strain of C. botulinum for each food product to be investigated. This is accomplished with a relatively large scale inoculated pack screening study involving nearly a dozen representative strains over a range of radiation levels.

2. Establishment of the microbiological safety of each irradiated food product artificially infected with the most radioresistant strain. This involves the use of a pilot plant size experiment, employing thousands of cans of food inoculated with the above selected organism.

3. Statistical evaluation of the radiation destruction data, obtained from the above two steps, in order to derive D values and safe radiocanning processes.

In addition to inhouse studies on radiocanning processes, applied radiation microbiology research has been concerned with such problems as the determination of the equivalence between radiation sterilization process and commercial thermal processes for cured meats, as well as the study of the incidence of C. botulinum in commercial grade raw meat products. Both of these problems are being studied by outside laboratories on a contract basis.

Two prototype inoculated-pack studies have been conducted to date, one with sliced canned bacon and another with chicken parts.

Bacon Study

The first inoculated pack bacon experiment has provided evidence that an irradiation dose of 4.5 Mrad insures the microbiological safety of the product. Two hundred cans of bacon inoculated with 6000 spores/g of C. botulinum strains 33A and 41B, respectively, and irradiated to 4.5 Mrad did not show any spoilage during four months of storage at 30°C. Furthermore, no viable organisms could be recovered from irradiated bacon.

On a smaller scale, it was also demonstrated that doses of 2.0 to 4.0 Mrad were sufficient to sterilize bacon inoculated with 6000 spores/g of C. botulinum. A total of 200 cans of bacon were irradiated at levels of 2.0 to 4.0 Mrad in 0.5 Mrad increments. None showed any swelling, and none contained recoverable viable organisms at the end of four months of storage at 30°C. Doses of 1.0 and 1.5 Mrad were not sufficient to destroy spores of C. botulinum 33A in bacon; whereas, neither spoilage nor toxicity was demonstrated for strain 41B (Table 2).

TABLE 2

Results from the Inoculated Pack Irradiated Bacon Incubated 4 Months at 30°C. Experiment I.

Mrad	No. of Cans per Strain of C. botulinum	C. botulinum Strain 33A Visible Swelling	C. botulinum Strain 33A Toxic Cans	C. botulinum Strain 41B Visible Swelling	C. botulinum Strain 41B Toxic Cans
0	20	12	8	4	0
1.0	20	1	5	0	0
1.5	20	0	1	0	0
2.0 to 4.0	a	0	0	0	0
4.5	100	0	0	0	0

[a] 20 cans at each dose between 2.0 and 4.0 Mrad at 0.5 Mrad increments for each strain, C. botulinum 33A and 41B. Each 300 x 200 can contained approximately 100 g of bacon and was initially inoculated with 6000 spores/g of C. botulinum.

Under the conditions of the above experiment, 2.0 Mrad radiation dose seems to be adequate to warrant the microbiological safety of the product. However, if the 12-D concept is adhered to, the minimal radiation dose can be estimated as follows:

1. From the data in Table 2 a D value for the strain 33A is 0.19 Mrad (using the equation of Schmidt and Nank).[21]

2. The initial total population is approximately 10^7 spores (100 cans; 100 g/can; 6 x 10^3 spores/g).

3. Hence, the minimal radiation dose can be estimated in two ways:

   (a) 12 x D
   12 x 0.19 = 2.3 Mrad.

   (b) 2 + (4 x 0.19) = 2.8 Mrad, where 2 is the experimentally determined minimal dose for an 8-log cycle reduction (10^7 to 10^{-1} spores); 4 is the additional number of log cycles to achieve the 10^{-12} reduction level of the initial population (10^7 to 10^{-5}).

A theoretically safe radiation sterilization process for canned bacon is, therefore, 1.0 Mrad higher in dose than demonstrated by actual experiment.

On the basis of data in Table 2 and the calculations derived from these results, a second bacon experiment was designed to provide information on (a) radioresistance in bacon of an additional 4 strains of C. botulinum (36A, 12885A, 9B, 53B) and (b) the safety of doses below 4.5 Mrad for sterilization of bacon. Four hundred cans were inoculated with about 3 x 10^4 spores/g with each strain, irradiated at dose levels of 1.5 to 3.0 Mrad in increments of 0.5 Mrad, incubated for 8 months at 30°C, and examined for spoilage, toxicity, and survival. Final data have not yet been completely evaluated. The experimental design and results to date are summarized in Table 3.

TABLE 3

Results from Inoculated Pack Irradiated Bacon
Incubated 8 Months at 30°C. Experiment II.

Mrad	Total No. of Cans Inoculated	No. of Swollen Cans
0	40	3[a]
1.5	400	1[b]
2.0	400	0
2.5	400	0
3.0	400	0

[a]Swelling occurred in 1 can/strain (viz., 36A, 12885A, 9B).

[b]Swelling at 1.5 Mrad was caused by strain 12885A. No swelling occurred in cans containing the other three strains.

Experiments clearly indicate that canned bacon is generally not easily attacked by C. botulinum while a potential danger of C. botulinum spoilage appears to exist in unprocessed raw canned bacon. Irradiation by as little as 2.0 Mrad may be sufficient for preservation of canned bacon under prevailing commercial conditions. The experiments also indicate a basic physiological difference between strains 33A and 41B; the latter was not able to grow even in unirradiated control cans of bacon. This observation probably represents differences between individual strains of C. botulinum. The factors affecting growth and toxin production of the various strains of C. botulinum in bacon are poorly understood and need more study.

Extrapolation from the available data to the 12-D kill indicates that 3.0 to 4.5 Mrad is statistically a safe radiation sterilization dose for bacon even under conditions of exaggerated artificial contamination with highly radioresistant spores of C. botulinum.

Chicken Pack Study

An inhouse microbiological study on irradiated canned chicken has been initiated to provide data for petitioning FDA to clear this product for human consumption. The study consists of two phases. Phase I is concerned with the selection of the most radiation resistant strain of C. botulinum. A total of ten strains (5 type A strains and 5 type B strains) is being screened for their radiation resistance in canned chicken. Phase II will be concerned with the establishment of the minimal radiation sterilization dose.

Phase I. Canned chicken parts (thighs and breasts) were inoculated with about 10^5 spores per gram of C. botulinum with each of the following strains: 33A, 36A, 62A, 77A, 12885A, 9B, 40B, 41B, 51B, and 53B. Twenty replicate cans per strain were irradiated to 0, 0.5, 0.8, 1.1, 1.4, 1.7, 2.0, 2.3, 2.6, 2.9, 3.2, 3.5, 3.8, and 4.1 Mrad and incubated at 30°C. Periodic observations are being made for swelling. Swollen cans will be examined for toxin and viable C. botulinum. Non-swollen cans will be checked for toxin and viable C. botulinum after six months of incubation.

Phase II. The following protocol is considered for Phase II of the inoculated chicken pack experiment:

		Most Resistant Strain	
Mrad	Cans	Spores/can	Total Spore Load
0	20	10^7	2×10^8
2.0	20	10^7	2×10^8
2.5	100	10^7	10^9
3.0	100	10^7	10^9
3.5	100	10^7	10^9
4.0	100	10^7	10^9
4.5	100	10^7	10^9

Cold Storage Study

An inhouse study was initiated to determine the effect of pre- and post-irradiation cold storage on the radioresistance of C. botulinum spores in canned chicken.

Commercial thermally processed chicken contained in 300 x 200 cans were infected with about 10^7 spores per can with strain 33A. Two sets of cans were stored at 40°F for 15 and 30 days, respectively, followed by irradiation to various dose levels and incubation at 86°F (30°C). Three additional sets of cans were irradiated first; one set was immediately incubated at 86°F; the remaining two sets were stored at 40°F for 15 and 30 days, respectively, prior to incubation at 86°F. All cans were observed for swelling at periodic intervals. Results after three weeks of incubation are summarized in Table 4.

TABLE 4

Effect of Pre- and Post-irradiation Cold Storage on Radioresistance of C. botulinum 33A Spores in Commercial Thermally Processed Canned Chicken

Mrad	Number of Swollen Cans During Incubation (86°F[a]) After Cold Storage (40°F)				
	No Cold Storage	Cold Storage Before Irradiation		Cold Storage After Irradiation	
		15 Days	30 Days	15 Days	30 Days
1.7	10	10	10	10	10
2.0	10	9	4	9	10
2.3	10	0	0	7	5
2.6	1	0	0	1	0
2.9	1	0	0	0	0
3.2	0	0	0	0	0

[a]3 weeks of incubation.

Both pre- and post-irradiation cold storage decreased the radioresistance of C. botulinum spores. Although pre-irradiation cold storage was the most effective treatment in reducing the sterilizing dose, this practice is questionable from the standpoint of microbiological safety. Prolonged periods of cold storage appear to be more desirable than short holding periods as a means of reducing the sterilization dose.

The above data suggests that cold storage treatment adversely affects the spores in a manner not yet understood. This phenomenon warrants additional investigation.

Equivalence of Radiation and Commercial Thermal Process for Cured Meats

A study entitled "Determination of equivalence between radiation sterilization process and commercial thermal process for cured meats" is being conducted by Swift and Company.

Canned cured ham was inoculated with a mixture of *C. botulinum* strains 33A and 41B at levels of 2.5 to 250,000 spores per gram of meat in 10-fold progressions. The cans were thermally processed to $F_0 = 0.2$, a commonly used commercial process, and incubated at 80°-100°F for six months. Toxic spoilage occurred with spore levels of 25,000 per gram and higher.

A second inoculated pack was prepared using spore levels of 2,500 and 250,000 per gram and irradiated to 0, 0.5, 1.5, 2.5, and 3.5 Mrad. Incubation at 80°-100°F is in progress. The cans will be examined periodically for spoilage.

Incidence of C. botulinum in Raw Foods

A second study by Swift and Company has just been initiated under the title "Incidence of *C. botulinum* in raw meats." Data are not yet available.

Viruses and Rickettsiae

A literature review just completed by McCrea and Horan of Quantum, Inc., indicates that viruses may be transmitted by foods. Clear-cut evidence for the occurrence of viruses either as primary contaminants (diseased animals) or secondary contaminants (introduced during handling) may be found in the literature. The epidemiological agents of Q fever, Russian tick-borne complex, foot-and-mouth disease, and rabies have been isolated directly from foodstuffs originating from diseased animals. Polio virus was isolated directly from foodstuffs handled by infected persons, and infectious hepatitis appears to be transferred by the fecal-oral route with food being a probably intermediate carrier. Presumptive evidence is available for infections from diseased animal products for cowpox and from secondarily contaminated foodstuffs for viral epidemic diarrhea, lymphocytic choreomeningitis, smallpox, and vaccinia.

No direct studies on the radiation resistance of food borne viruses are presently available. General studies on viruses indicate that very small viruses such as foot-and-mouth disease, polio virus, and presumably infectious hepatitis may be highly resistant to ionizing radiations, whereas large viruses such as herpes simplex or vaccinia may be less resistant. On the basis of their occasional occurrence in foods and their high radiation resistance, consideration must be given to the fact that viruses may present a potential public health hazard in foods processed by irradiation. It is true that

many of the viruses will be killed by the heat treatment required to inactivate enzymes. However, some viruses, particularly that of infectious hepatitis, may be rather heat resistant and may not be killed by the mild heat treatment required for inactivation of enzymes.

Microbiological Control

Taste test protocol requires that every can of irradiated food to be tested must be free of toxin of C. botulinum. Thus, mouse toxin tests as well as SPC's and tests for the presence of viable anaerobes were made on all irradiated foods. Thousands of samples have been tested since the initiation of the program, but in no instance has toxin been found. Spoiled samples receiving a dose of 4.5 Mrad have been recovered in a few instances of flexible packaged irradiated foods; this has been suspected to be due to pinhole failure of the package during handling and storage.

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TROOP ACCEPTANCE TESTS OF IRRADIATED FOODS

Elie Weeks

Introduction

First, I shall review briefly the two irradiated food tests conducted by the Quartermaster Field Evaluation Agency (FEA) at Fort Lee, Virginia, in 1958. Next, I'll describe the methods used by us after troop testing was resumed in June 1963 and give you the results to date of the two tests conducted so far this year. I'll close by telling you what I know of our plans for testing irradiated foods through 1968.

Initiation of Troop Testing in 1958

During April and June 1958, the FEA conducted its first troop test of irradiated foods (roast pork and bacon).[1] Prior to this time, government, industry, and many of our educational institutions had conducted tests of irradiated foods by feeding them to various types of animals and a few humans, including conscientious objectors. These experiments were so promising that The Surgeon General of the Army authorized the initiation of troop feeding tests in order to permit determining the relative acceptability of irradiated foods as compared with their fresh counterpart.

Testing Protocol of The Surgeon General in 1958

Medical Safeguards

Prior to clearing certain foods for troop testing, the Office of The Surgeon General established safeguards in the form of test protocols. These provided that:

1. All troops participating as test subjects had to have a complete physical both prior to and approximately three months after eating irradiated food.

2. Test participants had to wait three days between eating meals containing irradiated food.

3. Each can of irradiated food had to be given the following microbiological tests prior to being fed.

a. Mouse exotoxin assay (Clostridium botulinum).

b. Total plate count (aerobic bacteria).

c. Test for Salmonella.

d. Test for anaerobes.

4. The microbiologist performing these tests, the Quartermaster contract project officer, and a doctor representing The Surgeon General were required to sign a statement that all the cans of irradiated food had successfully passed all tests and were safe for human consumption. In addition, the Veterinary Officer gave his verbal approval to serve the contents of each can.

Congressional Interest

A senator, now deceased, expressed interest in the test which resulted in a requirement that all test participants must be volunteers, and that prior to volunteering they must have a thorough briefing on the irradiation preservation process. All who volunteered to participate were urged to write home and tell their families.

First 1958 Troop Test

Test Design

Three test sessions were held during each of which 120 men were fed a complete meal in an Army mess hall. Half (60) ate irradiated food and half ate its standard counterpart. The irradiated foods served at the three sessions were as follows:

1. Barbecued roast pork (sterilized).

2. Roast pork with gravy (sterilized).

3. Breakfast bacon (sterilized).

The irradiated pork had been stored in #10 cans at room temperature for ten months prior to testing. The bacon had been stored for 12 months. The standard counterparts were obtained from the Post Commissary. Acceptability ratings for the irradiated foods or their standard counterparts were obtained from each test subject, together with ratings for three other standard foods served at the same meal. This questionnaire, and all questionnaires used to date, have used the 9-point hedonic scale developed by the Armed Forces Food and Container Institute.

Test Procedures

Briefing Test Subjects. Troops from most of the large organizations at Fort Lee were briefed, during one of their weekly Information and Education sessions, on the background of the test and what they would be required to do as participants. Only 16 per cent of those oriented volunteered to participate. Many shied away from having to take another physical examination; others were alarmed that a physical examination was required both before and after testing. We, at the FEA, felt fortunate that 16 per cent of 1,975 troops oriented were willing to participate.

Physical Examinations. Some 14 volunteers were posted away from Fort Lee, departed on emergency leave, or got cold feet before they could be given their physical examinations. Of the 302 examined 45, 15 per cent, failed to be certified as eligible for testing.

Microbiological Assay. All the cans of irradiated food passed their laboratory examinations with flying colors. All microbiological assays were negative.

Feeding Troops. The test participants were collected from their widely scattered and numerous company areas and brought to a central mess hall. Half were fed irradiated food and half its standard equivalent. The secretly coded questionnaires indicated whether a test subject had been fed irradiated food or not. Questionnaires were dated and signed so the Hospital could know whether a man, going on sick call, had eaten a particular irradiated food.

Test Results

Acceptability. Statistical analysis of the test data showed that there was no significant difference between any of the irradiated foods and their standard counterparts. There is evidence that the level of all food ratings was higher than normal by approximately one point on the 9-point hedonic scale. National and local publicity regarding this test on irradiated food, both prior to and during the test, was such that the attitude of the individuals who volunteered could have been favorably influenced to the point of being atypical. These ratings were high for both irradiated and unirradiated foods; therefore, it is assumed that the difference between ratings of the experimental foods and their controls was not materially affected.

Table 1 shows the average hedonic scale ratings of the three irradiated foods and their controls together with the ratings of comparable unirradiated foods obtained during a pretest, conducted prior to the irradiated food publicity and under normal conditions of preparation, serving, and eating in the soldiers' own mess hall. Table 1 shows the very small and insignificant difference between the irradiated and unirradiated meats, and the high level of the test ratings compared with those of the pretest. Pretest averages were

TABLE 1

Average Hedonic Scale Ratings of Irradiated and Non-Irradiated Foods (Test #1 - 1958)

Menu Items Rated	Average Hedonic Ratings Irradiated	Control	Pretest
Barbecued Pork	7.80	7.82	
Roast Pork with Gravy	7.82	7.98	
Oven Fried Bacon	7.32	7.37	6.14
Mashed Potatoes		7.57	6.72
Green Beans		7.52	5.96
Carrots		7.27	5.39

based on more than 300 individual ratings; test averages on 60 ratings. The nine-point hedonic scale assigns a value of nine to the rating "Like Extremely," eight to the rating "Like Very Much," down to one for the rating "Dislike Extremely."

Plate Waste. Data on plate waste were inconclusive. This was primarily due to differences in fat content of the irradiated and standard pork. Since practically all plate waste was fat, these data could be interpreted less as a measure of acceptability than as a measure of relative fat content. There was no significant difference in the bacon plate waste.

Post-Test Physical Examination of Test Participants. Physical examinations were given to 109 of the 139 soldiers present at Fort Lee three months after eating irradiated food. These examinations disclosed no effects which could be attributed to the consumption of the irradiated foods.

Second (1958) Troop Test

Foods Tested

During November and December 1958 a second test[2] of six irradiated foods was conducted. Four foods were sterilized and two pasteurized; they were then stored at room temperature for 90 days prior to testing. The foods were:

1. Chicken parts, fried (sterilized).
2. Shrimp, deep fat fried (sterilized).
3. Carrots, diced (sterilized).
4. Chicken stew (sterilized).
5. Fruit compote (pasteurized).
6. Pineapple jam (pasteurized).

Protocol

The same testing protocol was followed with the minor exceptions that the second physical could be given after six weeks instead of three months and that a pasteurized food could be fed, during the same meal, to a test participant fed a sterilized food. The change from three months to six weeks enabled the FEA to use as test subjects Quartermaster School troops stationed at Fort Lee while taking their MOS training. This provided a much broader base for selection and made it much easier to collect the men for each test session. The permission to feed sterilized and pasteurized irradiated foods to the same man at the same meal was not utilized in this second test.

Test Design

Two separate sets of 100 plus test subjects were used for each of two identical replications of three meals. The men in the first replication were subdivided into two groups of 50. One group served as the control for the other, as shown in Table 2. The second replication was identical to the first, except the men were from different school classes, and the three test sessions were held almost a month later

TABLE 2

Feeding Test Design, First Replication (Test #2 - 1958)

Session No.	Group #1	Group #2
1	Chicken stew, standard Fruit compote, irradiated	Chicken stew, irradiated Fruit compote, standard
2	Fried chicken, irradiated Diced carrots, standard	Fried chicken, standard Diced carrots, irradiated
3	Shrimp, irradiated Pineapple jam, standard	Shrimp, standard Pineapple jam, irradiated

Test Procedures

The test procedures were the same as those used in the first irradiated food tests. The same briefing was followed; the pre- and post-physical examinations were conducted; the cans of food were each examined and tested under the same microbiological assay protocol prescribed by The Surgeon General; the same feeding procedures were followed including obtaining acceptability ratings on four food items, one of which was either irradiated or an unirradiated equivalent. The test subjects were not told which foods had been irradiated.

Test Results

Acceptability. There were no significant differences (5% level) between the average ratings of irradiated chicken stew, fruit compote, and pineapple jam and their standard equivalents in unirradiated foods. However, the average ratings of irradiated fried chicken, shrimp, and diced carrots were significantly lower than their controls (see Table 3).

TABLE 3

Comparative Average Hedonic Scale Ratings for Irradiated and Standard Foods Combined Over Both Replications (Test #2 - 1958)

Food Item	Irradiated		Standard		Statistical Significance[a]
	Number Subjects Rating	Average Hedonic Rating	Number Subjects Rating	Average Hedonic Rating	
Chicken stew (chicken pot pie)	107	7.37	104	7.58	N.S.
Fruit compote (spiced fruit pie)	104	6.90	107	6.74	N.S.
Chicken parts (fried chicken, Maryland style)	101	7.38	103	7.95	Sig.
Diced carrots (parsley buttered carrots)	103	3.66	101	7.02	Sig.
Shrimp (deep fat fried)	107	6.95	101	7.69	Sig.
Pineapple jam	103	7.33	107	7.49	N.S.

[a]N.S. Difference between mean ratings is not significant at the 5 per cent probability level.

Sig. Difference between mean ratings is significant at the 5 per cent probability level.

Except for the diced carrots the high level of the irradiated food ratings would indicate that they are sufficiently well liked to justify serving them as part of the standard Army garrison ration.

Plate Waste. Only in the case of diced carrots was there a significant difference between irradiated food plate waste and the unirradiated control

food—57.5 per cent of the irradiated diced carrots servings were left uneaten compared with 18.3 per cent of the control.

Post-Physical Examinations of Test Participants. Of the 224 troops who consumed irradiated food during this test, 174 were present at Fort Lee to complete the post-physical examination six weeks after participating in the test. These examinations revealed no effects which could be attributed to the consumption of the irradiated foods.

Laboratory Tests. The results of the laboratory tests for toxicity and microorganisms were negative in all instances.

Suspension of Troop Tests

Early in 1959 The Surgeon General withdrew his clearance for troop tests of irradiated foods, and this portion of the radiation program remained suspended until 1963.

Troop Testing Resumed: First (1963) Test

Foods Tested

During the period 10 - 19 June 1963 troop testing of sterilized irradiated food was resumed with the testing of fried chicken, grilled pork chops, and oven fried bacon[3] which had been stored at room temperature for five months prior to feeding to troops.

Protocol

The Surgeon General's protocol was materially modified in a manner that made it possible to conduct troop tests with fewer administrative headaches. The following are the major conditions of the new protocol:

1. Physical examinations are no longer required. Now, a doctor, representing The Surgeon General, screens the medical records of the test participants to eliminate chronic complainers and those with a medical history of gastro-intestinal distress or other ailments which would make them unsuitable as test subjects. Based on this screening the doctor certifies those who may participate.

2. The Surgeon General has also agreed that for sterilized foods, irradiated at 4.5 Mrad, it is only necessary to demonstrate that they are free from any evidence of toxicity. Therefore, only a Mouse Exotoxin Assay for presence or absence of *Clostridium botulinum* toxin is required.

3. Also it is required that a Veterinary Officer certify that the food is wholesome and suitable for troop consumption; that cobalt-60 was the only source of radiation used and the cans had no residual radiation; and that the contract microbiologist and the Veterinary and Medical Officers must sign a statement that the foods are suitable for troop feeding.

Test Design

The experimental design was quite simple. Three companies were fed the three foods in each order over a three-day period. This procedure was then repeated so as to provide a replication. Half of the men who normally ate in the messes, i.e., did not ration separately, were fed the three meats in irradiated form (these were the men who had been certified by The Surgeon General) while the others were fed the standard control meats. During the first three-day test period, the men were not informed that they were testing irradiated food. During the second period they were briefed that irradiated food was being tested and that one of the four foods they ate and rated might be irradiated.

Test Procedures

A doctor from Kenner Army Hospital, Fort Lee, Virginia, serving as The Surgeon General's representative, examined the records of the troops assigned to the three messes who regularly ate in the company messes. When the doctor had determined that 50 per cent of these men in each mess could be certified to eat irradiated food, their names were furnished the test team and only these men were permitted to eat irradiated food. The others, including transient visitors and those on separate rations, were fed the unirradiated control.

The same animal assay procedures used in the previous tests were followed. The test foods were prepared and served as part of regular meals. Each test participant was required to rate, on the nine-point hedonic scale, four of the principal foods served, including the irradiated meat or its control. Plate waste was also obtained since this usually has a negative correlation with food acceptability; if it does not, a restudy of the test procedures and test data should be made.

Test Results

Acceptability. The average preference rating of the irradiated foods was sufficiently high for them to be considered acceptable components of standard garrison meals.

1. Pork Loin. There was no significant difference between the average ratings of irradiated and standard pork loin when served as pork chops.

2. Fried Chicken. The standard fried chicken was more acceptable than the irradiated chicken, but the difference in preference was sufficiently small and the level of acceptability of both sufficiently high to warrant considering both as acceptable for troop feeding.

3. Bacon. The standard bacon was more acceptable than the irradiated bacon. Comments by the test subjects and observations made by the test team warrants the conclusion that part of this difference can be attributed to the higher proportion of lean meat in the standard bacon.

Plate Waste. There was a high negative correlation between the acceptability of the individual food ratings and the percentage of plate waste. This confirmed the findings as to acceptability.

Prior Knowledge. During the second three days of testing, the troops were informed that they were participating in a test of irradiated foods and that one of the four foods they would be asked to evaluate had been preserved by irradiation. This knowledge had no effect on the level of consumer preference ratings during the second three days of testing. Table 4 shows the average hedonic ratings for the irradiated and standard equivalent foods for both sessions.

TABLE 4

Average Hedonic Ratings of Irradiated and Standard Foods, Week #1 and #2 (Test #1 - 1963)

	Week #1			Week #2		
Menu Items Rated	Irradiated	Standard	Sig.[a]	Irradiated	Standard	Sig.[a]
Pork chops	7.06	7.21	N.S.	7.27	7.28	N.S.
Chicken, fried	6.77	7.21	N.S.	6.66	7.18	Sig.
Bacon	5.62	6.52	Sig.	5.57	6.53	N.S.

[a]Sig. Difference between average ratings is significant at the 5 per cent probability level.

N.S. Difference between average ratings is not significant at 5 per cent probability level.

The averages shown in Table 4 are averages of company averages, not averages based on the total of all individual ratings. They were computed on this basis because of evidence that the companies differed significantly in the level of their ratings and the number of participants varied from company to company.

Second (1963) Test

Objective

Earlier tests had as their general objective the determination of whether troops would find a particular irradiated food acceptable when served as part of a garrison meal. The criteria of acceptability were:

1. How does it compare with its unirradiated standard counterpart?

2. Is the level of its rating on the 9-point hedonic scale sufficiently high, compared to alternate foods, to justify including it as part of the Army's ration system?

The second (1963) test had as its objective: To determine whether continued consumption of an irradiated food, chicken, served at least once a week over a period of a month, has any effect on consumer preference rating.

Test Design

Five test sessions were selected which corresponded with the frequency with which chicken appeared on the Master Menu. The same soups, vegetables, beverages, and desserts called for by the Master Menu were served with both irradiated and unirradiated chicken. The recipes were:

1. Southern fried chicken (sterilized).
2. Oven fried chicken (sterilized).
3. Barbecued chicken (sterilized).

The irradiated and unirradiated chicken were served to the troops eating in six Fort Lee messes in a design requiring each recipe to be eaten an equal number of times during the test. Half of the regular members of each mess were designated (after inspection of their medical records) to eat only irradiated foods. The other half, plus transients and those rationing separately, were fed only the standard, unirradiated chicken. All questionnaires were coded and signed to assure that the right food was served to the right man and to permit our statisticians to keep track of those individuals who ate chicken at the five test sessions. This permitted eliminating from tabulation any transients or men rationing separately. Such men usually eat only the noon meal in their company mess and eat their other meals at home.

Test Procedures

The same briefing of cooks and test participants was given as in the previous 1963 test. Also, the same laboratory procedures were followed. As the test participants approached the mess serving line they were checked against The Surgeon General's list and given the properly coded questionnaire to assure their receiving the correct test item. Each test subject rated four

designated foods and was never aware of whether he was receiving irradiated or unirradiated foods. Plate waste data were not obtained during this test because of a shortage of test team personnel, and the fact that these data had already been obtained in previous tests of chicken. The test sessions were held on:

13 September	-	Friday
17 September	-	Tuesday
24 September	-	Tuesday
1 October	-	Tuesday
4 October	-	Friday

On each date, three messes had test sessions at dinner and three at supper meals. The next and subsequent test days, the messes alternated dinner and supper sessions.

Test Results

Since the last test session was Friday, 4 October, the data had not been tabulated prior to my departure from Fort Lee.

Third (1963) Test

Objective

This test has as its objective the determination of relative troop preference for three meats preserved by radiation when served as part of a normal meal under garrison feeding conditions.

Test Design

The three foods to be tested are:

1. Ham, baked, boneless (sterilized).
2. Bacon, prepared from troop issue slab bacon (sterilized).
3. Haddock, fillets (pasteurized).

The design of test has not been firmed up, and the test session dates are still flexible; however, present plans are to conduct the test during late November and early December 1963.

Future Tests, FY 1964 Through FY 1968

The FEA has programmed to conduct two more irradiated food tests during FY 1964, one each in the 3rd and 4th quarters. We have been advised to prepare ourselves to conduct one irradiated food test each quarter thereafter through 1968.

You can see from this that we at FEA will be kept busy evaluating the progress made by the Natick Radiation Laboratory. Our hope is that by conducting impartial and statistically valid tests we can furnish them the information they need.

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PACKAGING FOR RADIATION STERILIZED FOODS

Eugen Wierbicki

Introduction

The main objective of the process of food preservation by ionizing radiation is to provide the Army with foods which would be as close as possible to the fresh, home-cooked varieties of foods, yet which could be stored without refrigeration for at least two years under adverse storage conditions.

Packaging is an important factor for achieving this goal. Many studies have been conducted on various packaging materials for radiation processed foods since the beginning of the Army Program for The Radiation Preservation of Foods. Metal cans, tin coatings, various internal enamels, sealing compounds, and flexible containers have been investigated for their physical, chemical, and protective characteristics under the impact of irradiation and post-irradiation storage while in contact with various foods.

I will review the past achievements made in the field prior to building the Radiation Laboratory at Natick and describe our present research and development work on packaging for irradiated foods.

Past Achievements

Reported in this section are the achievements made by the Container and Radiation Preservation of Foods Divisions of the Armed Forces Food and Container Institute when the Institute was located in Chicago. The activities of the Institute have recently been transferred to the U. S. Army Natick Laboratories, Natick, Massachusetts.

During the Seventh Contractors' Meeting of the U. S. Army's Radiation Preservation of Foods Program in Chicago, on 6-8 June 1961, Mr. George E. Tripp, who was at that time Chief of the Container Technology Branch of the Institute, gave a thorough review on the subject. The published version of his review is recommended as a key reference in the field prior to 1962.[1]

As a result of the past research and development work on the subject, we have available a tin can which is a completely reliable package for radiation sterilized foods.

The types of steel and weights of tin coating used in cans for thermally processed foods were found to be satisfactory for foods processed by sterilizing doses of ionizing radiation. Three types of can enamels, oleoresins, epoxy-phenolic, and polybutadiene were found to be satisfactory. A nearly universal enamel for any kind of irradiated foods, particularly radiation sterilized meats, is "C-enamel," which is an oleoresinous compound containing zinc oxide.

Several end-sealing compounds were found to be satisfactory also. Butadiene-styrene, butadiene-acrylonitrile rubber, and natural rubber, used as end-sealing compounds for tin-plate cans proved to perform even better after irradiation since they are crosslinked and toughened by irradiation. Aluminum containers also appear satisfactory for irradiated foods when used with the coatings mentioned for the tin cans.

The first radiation preserved food, fresh bacon irradiated with 4.5 Mrads, which was cleared by the U. S. Food and Drug Administration on 8 February 1963 for unrestricted consumption by the U. S. Public, was packed in a tin plate can coated with C-enamel.

The only major outstanding problem with the use of metal cans for packaging radiation sterilized foods is the gas evolution during radiation exposure which causes the loss of vacuum and may result in can swelling. Closing under vacuum and certain allowance for the head space in the can are the present measures which are used to counteract this undesirable side-effect of radiation.

However, this does not solve the problem of the gas production in irradiated foods. Basic research on the nature of head-space gases, as produced by irradiating different kinds of packaged foods, is presently in progress (American Can Co.). This is the first phase of a two-year research project designed for finding some means to prevent or drastically minimize the production of head space gases in radiation sterilized packaged foods.

In meeting our packaging requirements for military use, the metal cans, in spite of their many advantages, have some shortcomings, for example, heavy weight, bulky shape, high cost of manufacturing cans of new dimensions, dependence on the imports of tin. Therefore, a decision was made to develop flexible containers for packaging foods for the Armed Forces.

George Tripp, in cooperation with the packaging industry, laid the groundwork for developing flexible containers for irradiated foods.[1] Numerous commercially available plastic films and their laminates were investigated for their protective qualities and their resistance to radiation. The result was that there was no unqualifiedly suitable material for in-package irradiation of foods. However, encouraging data were obtained which indicated that flexible packaging for irradiation processed foods might be developed after a more thorough and systematic investigation of the problem. For example, it was found that polystyrene may be useful as a package for non-fatty foods; one type of polyvinylidene chloride was satisfactory for partially-baked rolls; another type

was suitable for fresh pork, while a polyethylene-polyester combination film performed well with orange juice and whole irradiated hams. This, however, was true only from the standpoint of withstanding ionizing radiation within the range from one to six Mrads, without considering protective characteristics of the films needed for long-term storage of irradiated packaged foods under military supply and combat conditions.

Present Research and Development Work

The present work on packaging for irradiated foods is concentrated on developing flexible plastic containers for in-package radiation sterilization of various foods, with emphasis on radiation sterilized meats.

In order to accelerate the work, the Packaging Section has been established within the organizational structure of the Irradiated Food Products Branch, Food Division, with the sole responsibility of developing flexible containers for radiation sterilized foods and testing and improving the use of metal cans as the packaging material now available.

The Packaging Section is working in a close cooperation with the Container and Clothing and Organic Materials Divisions here at Natick and with the chemical and packaging industries, through contract research in specialized areas, based on mutual interest.

The initial work of the Packaging Section was to establish criteria for developing flexible containers for radiation sterilized foods. A review of the available reports on the subject revealed that irradiation causes a decomposition of plastic films forming undesirable odorous compounds and extractives and changes in physical properties of the films. The data also indicate that flexible packaging materials need to be improved in their resistance to microbial penetration, insect attacks, and various physical abuses under the military logistic system.[2]

Consequently, the objectives for developing suitable flexible containers for radiation sterilized foods have been formulated as follows:

a. The flexible containers must be resistant to changes to their protective characteristics (sealing, permeability, resistance to microbial and insect penetration, rough handling, creasing, stress, cracking, etc.).

b. They must not be adversely affected by radiation induced changes in the food.

c. They must not transmit adverse odors and flavors, or toxic extractives to the food.

d. They must be of such size and shape as to utilize the radiation energy most efficiently.

Based on the available information, it was concluded that at this time not a single commercially available plastic film would meet all these requirements. A combination of various films in the form of laminates or flexible pouches, by incorporating aluminum foil as the gas and vapor barrier, was considered to be the best approach to solving the problem.

The assistance of the National Academy of Sciences—National Research Council and packaging industry was solicited. A research contract for developing flexible containers was offered to the industry and competent research institutes. The proposed contract work has been divided into two segments covering the following phases.

a. Determination of the extractives and other fragmentation compounds of various food packaging polymeric materials produced by ionizing radiation.

b. Development of flexible containers that will have all necessary chemical, physical, and protective characteristics that will meet the military requirements.

The response to this research offer was very great, and we appreciate the cooperation and interest of our many friends in the packaging industry and in various research establishments.

After a thorough review of the proposals, a research contract was awarded to the Continental Can Company to work on the problem.

I will review the first phase of the work which is now in progress. It is concerned primarily with the determination of the extractable substances which may be formed by irradiation of various plastic materials.

Table 1 presents the food contacting materials used in this research. It covers six plastic films, each different in its chemical and molecular structure, which are commonly used as food packaging materials. Cellophane has been excluded from the study since cellulosic materials have a low resistance to radiation above 1.0 Mrad.[3]

The following approach was used for evaluating the selected packaging material. The films, each 1.0 mil in thickness, were laminated to 0.5 mil aluminum foil, covered with 32 lb pouch paper (Table 2).

The resulting laminates were formed into pouches, approximately 4-1/2" x 7", with 3/8" seals on three sides, while leaving an opening on the edge of the smaller dimension of the pouches. The capacity of the pouches is approximately 5 to 5-1/2 fluid ounces of a product.

At this state of research, the aluminum foil and the paper cover are used merely as protective materials for the food contacting films. Yet, the resulting experimental pouches simulate the projected future use of the plastic films, which might be selected as the food contacting materials in the final

TABLE 1

Food Contacting Material

Material Code	Generic Name	Trade Name[a]	Supplier
A	Polyethylene (MD-0.930)	Petrothene 239	U.S.I.
B	Polyvinylidene Chloride Copolymer	Saran 18L	Dow
C	Polyvinyl Chloride (Unplasticized)	Bakelite 3027	Cadillac
D	Polyester	Mylar A	DuPont
E	Polystyrene	Polyflex	Plax
F	Nylon 6	Capran C	Allied

[a]The mention of the trade names in this table does not constitute endorsement of the products used over comparable materials.

TABLE 2

Pouch Specifications

Structure:

Paper	Polyethylene	Al Foil	Food Contacting Films
32#Pouch	0.75 mil	0.5 mil	1.0 mil of A, B, C, D, E, or F

Volume: App. - 5 fluid ounces

Dimensions: 4-1/2" x 7" with seals 3/8" on three sides. The opening is on the edge of the smaller dimension.

version of flexible containers for irradiated foods. The dimensions of the pouches are such that they are sufficient for packaging Ready-to-Eat individual meals and the manufacturing of the pouches can be made by commercially available pouch forming and vacuum sealing equipment. In this way, while investigating the formation of extractives from the films by irradiation, we

may obtain at the same time valuable practical data for developing final versions of flexible packaging for irradiated foods, planned for the next phase of the over-all project.

The pouches will be filled with five ounces of food simulating solvents, representing neutral, acid, fatty, and salty foods (Table 3), then sealed at their opening ends. At the outset of this research program, it was realized that it would be impossible to determine the amount and chemical identity of the film extractives which may migrate into actual foods since food products are of a very complex chemical composition and may contain some components that could react and interfere with the identification of the organic migratory substances released by the films on irradiation. Therefore, the use of the food simulating solvents is being used instead. This approach is presently used by packaging industry for obtaining the necessary clearances from the Food and Drug Administration for new varieties of food packaging materials.[4]

TABLE 3

Food Simulating Solvents

Solvent	Simulated Foods
Demineralized distilled water	Neutral foods (Meats)
Acetic acid solution, pH 3.5	Acid foods
Freshly redistilled n-heptane	Fatty foods
3.5% NaCl, 0.5% Na-phosphate solution, pH 6.2	Salty foods (Cured meats)

In order to provide physical protection for the pouches, filled with the food simulating solvents, during shipment to the irradiation source and return, each pouch is enclosed in a paperboard jacket, bonding the pouch to one panel of the jacket. The pouches are then arranged in a cardboard shipping carton in such a way that they overlap each other to provide a more uniform dose distribution during irradiation. The shipping carton contains 48 pouches arranged in two tiers of 24 pouches each, with three rows of eight pouches per tier.

Table 4 gives the dimensions of the protective jackets and the shipping cartons used in this investigation. Figures 1, 2, and 3 present external views of the flexible package and protective jacket and the arrangements of the pouches in the shipping carton, respectively.

A study of the dose distribution within the shipping carton, filled with the experimental pouches, has been completed by Mr. Robert D. Jarrett of our Radiation Sources Branch. The results indicate that the required dose of 6.0 Mrads of gamma radiation was attained within the pouches with about 10 per cent greater dosage of the absorbed radiation at the edges of the pouches.

TABLE 4

Jacket Specifications

Material: Solid sulfate board, 16 point (PPP-B-566)

Outside Dimensions (Folded): 4-3/4" x 7-1/4" x 1/2"

Shipping Carton Specifications

Inside Dimensions: 12-1/2" x 5-1/4" x 15"

No. of Pouches Per Carton: 48 (2 layers of 24 each)

The use of the paperboard jackets was made not only for the purpose of protecting the experimental pouches during shipment to the radiation source and return, but also to demonstrate an actual use of the flexible containers in the future, for example, the case of Ready-to-Eat individual meals. Realizing the type and degree of rough handling under the military supply system, an external protection for the irradiated foods packaged in flexible containers will probably be required.

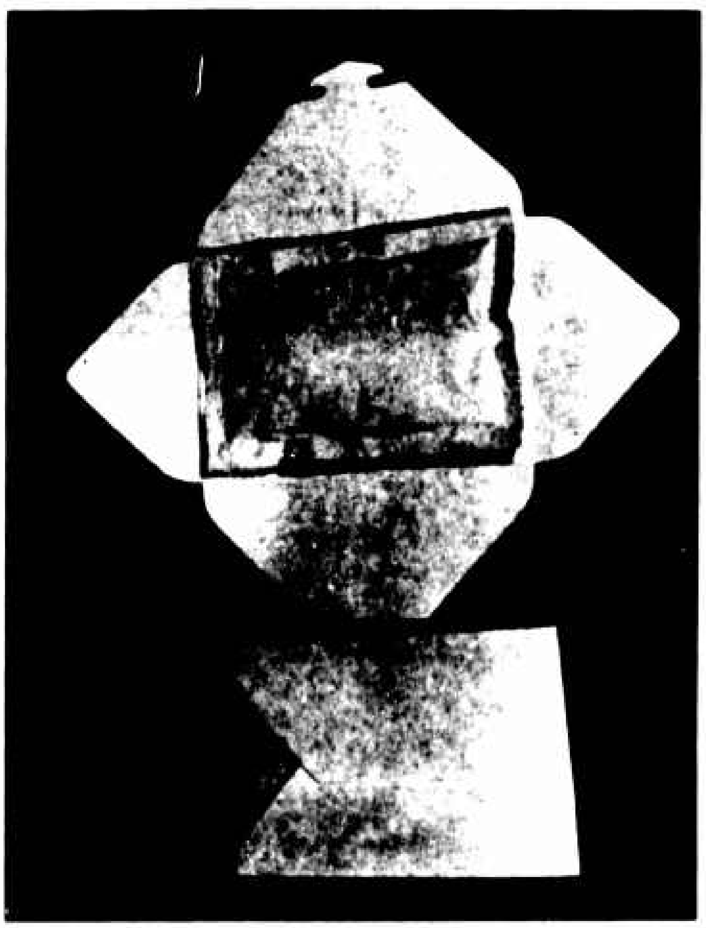

Figure 1. Flexible package and protective jacket.

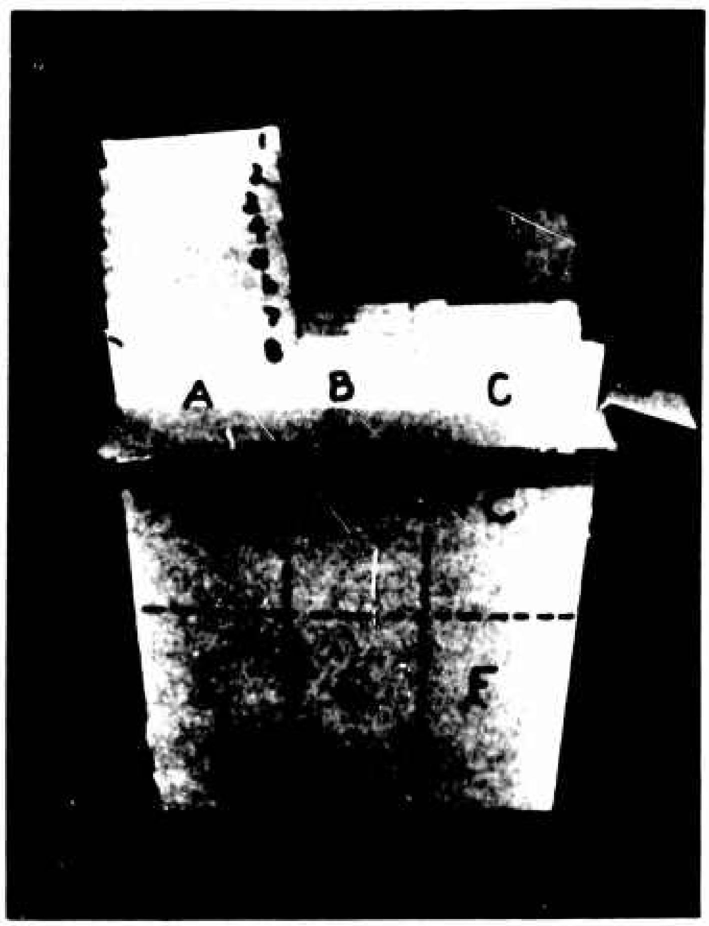

Figure 2. Side view of pouches in shipping carton.

The usefulness of such protective jackets have already been demonstrated by the Container Division of the U. S. Army Natick Laboratories in the case of flexible packages for thermally processed foods. The same could be said for the shipping cartons, which can eventually be used for shipping 48 pouches for irradiation as well as for their military distribution after irradiation and storage without the necessity of removing and repackaging the pouches after irradiation.

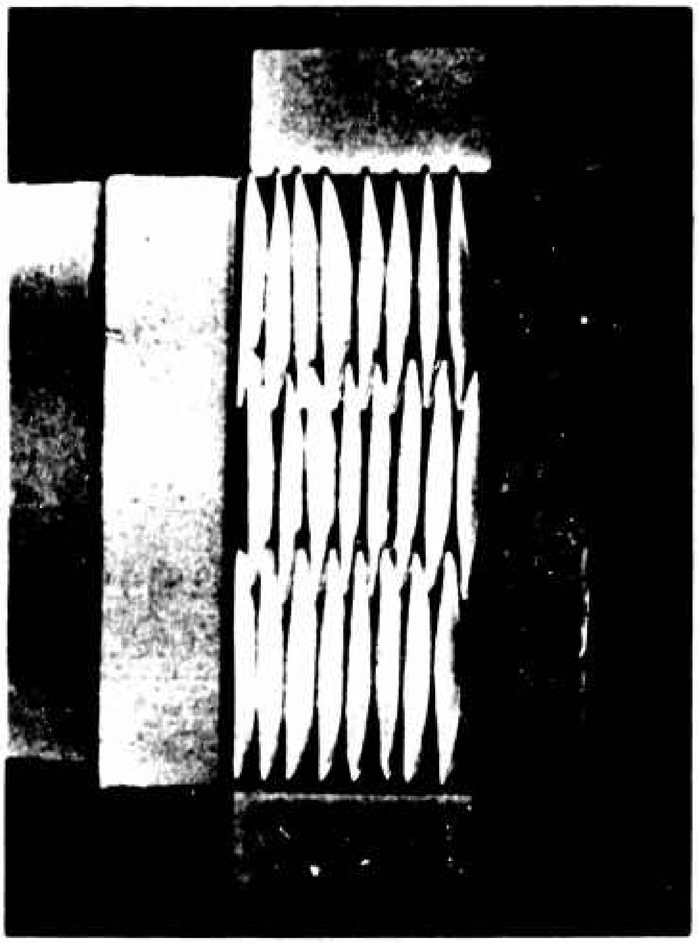

Figure 3. Top view of pouches in shipping carton.

Figures 4 and 5 show further steps of the experimental procedure after the pouches are filled with the food simulating solvents and irradiated.

The irradiation dose of 6.0 Mrads is 1.5 Mrad higher than the required sterilizing dose for many irradiated foods. The control (0.0 Mrad) samples are being used parallel with the test samples to determine the extractives which might be released by the packaging films into the food simulating solvents without irradiation. The control and the test samples will then be stored for six weeks at 100°F and 50 per cent relative humidity, or for one week in case of n-heptane. After completion of the storage requirements, the food simulating solvents will be removed and the extractives determined as water soluble and chloroform soluble fractions. The combined amount of the extractives will be reported in terms of the total and chloroform extractable material per unit area of the food contacting films. The nature of the extractives will be determined by infrared spectroscopy of the chloroform soluble extractives. In case of heptane, infrared spectroscopy will be run directly on the solvent after irradiation and storage, while in contact with the films.

In addition to the extractives determination, additional information, which will be useful for the Phase II of the over-all project for development of flexible containers for irradiated foods, will be obtained on the experimental films during this investigation. These additional data will include odor and color of the food simulating solvents, the incidence of leakage and the seal strength, brittleness, and appearance of the food contacting films after irradiation and storage.

Inasmuch as the knowledge of the extractives formation by food contacting films under the effect of sterilizing doses of ionizing radiation is

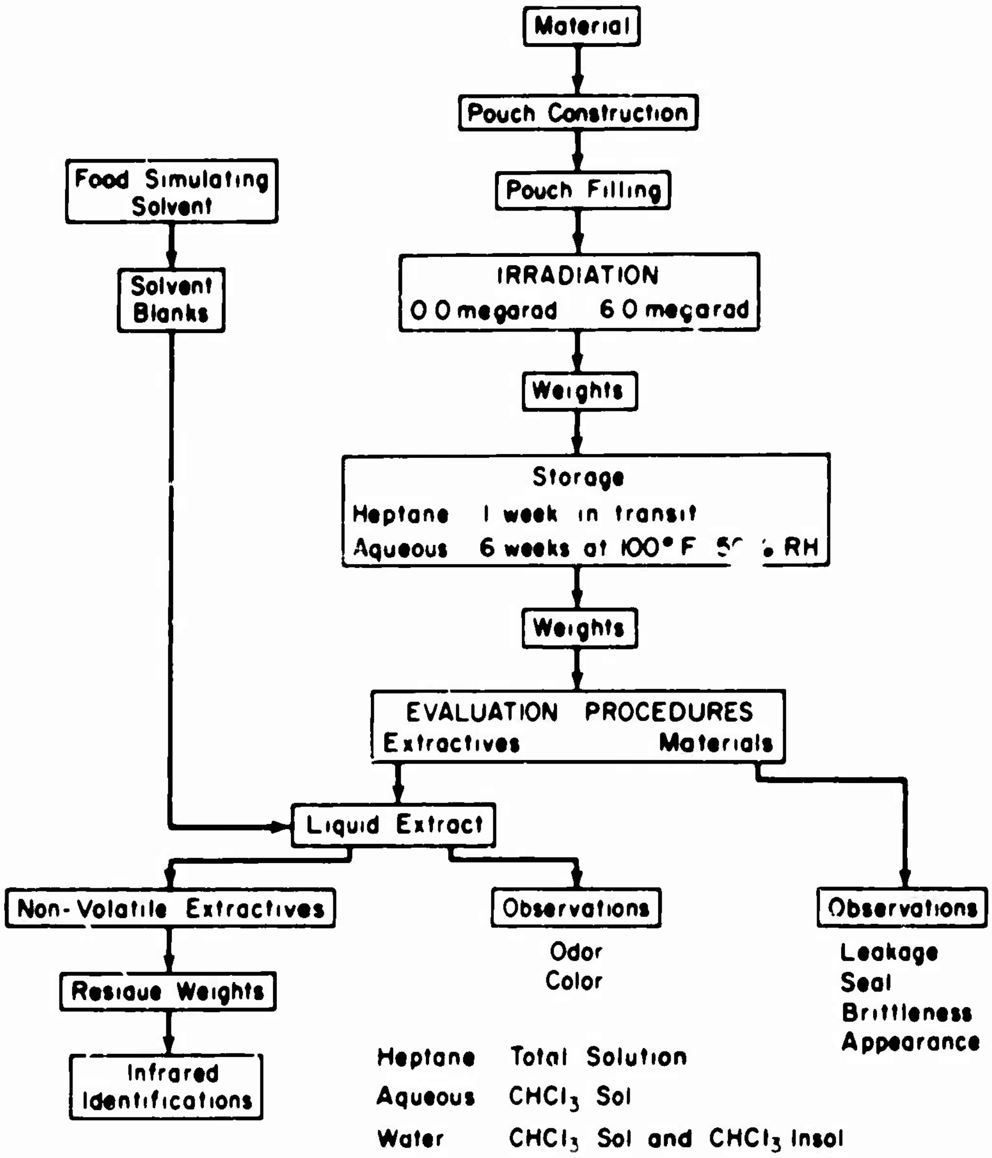

Figure 4. Extractives procedures.

I.	Aqueous Solvents	
	Pouches per sample (one dose, one solvent)	5
	Samples per dose level (one solvent)	4
	Total pouches per dose (one solvent)	20
	Dose levels (0, 6 Mrad)	2
	Total pouches for one solvent	40
	Solvents (pH approx. 7, 6.2, 3.5)	3
	Total pouches	120
II.	Heptane	
	Pouches per sample (one dose)	2
	Samples per dose	4
	Total pouches per dose	8
	Dose levels (0, 6 Mrad)	2
	Total pouches for all doses	16
III.	Total number of pouches per material	136

Figure 5. Pouch requirements for each material; 5 oz. pouches (150 ml, approximately 50 sq. in. surfaces).

a key prerequisite for selecting films for flexible containers, additional research on plastic films extractives was performed. Three prototype commercially available plastic laminates were selected for this investigation. In addition, one transparent film, not being investigated by the Continental Can Company, was added to the list of the experimental material. The work was performed by the Hazleton Laboratories, a contractor for the Atomic Energy Commission on the extractives study of the packaging materials intended for use with radiation pasteurized foods.

This research was performed as a joint effort with the AEC and the technical approach to the evaluation of the films was generally the same as used by the AEC, with the exception of the irradiation dose which was 6.0 Mrad instead of 1.0 Mrad used for radiation pasteurization treatment.

The following packaging materials* were investigated:

a. DuPont #15-Series R/0.5 mil polyethylene - 0.7 mil Al foil - 2.0 mil polyethylene.

b. 3M Paper-Foil-Scotchpack laminate, series #AX46, total thickness 5 mil.

c. Fabron Mylar-Foil-Vinyl laminate, consisting of 0.5 mil polyester - 0.35 mil Al foil - 2.0 mil polyvinyl chloride.

d. 3M Cryogenic KEL-F transparent film (5.0 mil amorphous-type chlorotrifluoroethylene polymer, series No. KX-8212).

The extraction study was performed by using the extraction cells developed by the Continental Can Company[3] with slight modifications by Hazleton Laboratories. Twenty-four cells were prepared for each material and divided into three groups of eight cells each. Each cell of one group contained 200 ml redistilled spectral grade heptane, each cell of the second group contained 200 ml deionized water, and each cell of the third group contained 200 ml of 0.01 N acetic acid (pH 3.5). Four cells from each group were exposed to 6.0 Mrads of cobalt-60 radiation while the remaining four served as non-irradiated controls. All cells were then stored for seven days at $70^{o} \pm 3^{o}F$ and 75 per cent RH ± 5 per cent RH.

The solvent from each cell was decanted through glass wool into a graduated cylinder and the volume recorded. The solvent was transferred with absolute ethanol washings into a beaker. Aqueous and acid extracts were then evaporated at 80°C in a drying oven with an attached oil-free nitrogen supply. Heptane extracts were evaporated on a hot plate at 40°C under a stream of oil-free nitrogen. The contents of four beakers, representing four cells treated in the same manner, were transferred with ethanol washings

*The mention of specific trade names throughout this paper does not constitute endorsement of the products used over comparable materials.

into a tared porcelain crucible and the solvent evaporated to dryness. The residue was dried for four hours at 110°C in an oven, cooled in a dessicator for one hour, and weighed again. This was repeated to constant weight, ±0.05 mg.

The residues from the aqueous and acid extract were extracted with five 10 ml portions of warm spectral grade chloroform, stirring well after each addition, and the extract filtered through a sintered glass filter into another tared porcelain crucible. The solvent was evaporated and the residue dried to constant weight as described above. The amount of extractives was calculated as mg/sq in., the area of exposed film in the cell being 85.6 sq in.

The total and chloroform soluble extractives are reported in Tables 5 and 6. The DuPont and 3M laminates (Table 5) have 2.0 mil polyethylene films as the food contacting materials. As the data indicates, 6.0 Mrad

TABLE 5

Extractives from Irradiated (6.0 Mrad) Food Packaging Material (AEC-Hazleton Labs., Inc.)

Material	Total Extractives (mg/sq in.)/solvent			CCl_3CH_3 Extractives (mg/sq in.)	
	Water	Acetic Acid	Heptane	Water	Acetic Acid
DuPont #15 Series R. Lam.*					
Irradiated	0.02	0.52	1.67	0.01	< 0.01
Control	0.04	0.03	0.16	< 0.01	0.01
Difference	-0.02	0.49	1.51	0.00	0.00
3M Paper Foil Scotch Pack Lam.*					
Irradiated	0.02	0.49	0.47	0.01	< 0.01
Control	0.03	0.02	0.25	< 0.01	< 0.01
Difference	-0.01	0.47	0.22	0.00	0.00
DuPont 125-A-101 Polyethylene (Irrad. 1.0 Mrad)					
Irradiated	0.20	0.25	0.20	0.01	0.02
Control	0.01	0.18	0.32	< 0.01	0.01
Difference	0.19	0.07	-0.12	0.00	0.01

*Food Contacting Films = Polyethylene

TABLE 6

Extractives from Irradiated (6.0 Mrad) Food Packaging Material (AEC-Hazleton Labs., Inc.)

Material	Total Extractives (mg/sq in.)/solvent			CCl_3CH_3 Extractives (mg/sq in.)	
	Water	Acetic Acid	Heptane	Water	Acetic Acid
Fabron, Inc. Mylar-Foil-PVC Lam.*					
Irradiated	0.06	0.58	2.22	0.01	0.01
Control	0.06	0.03	2.00	0.01	0.01
Difference	0.00	0.55	0.22	0.00	0.00
3M KEL-F (Chlorotrifluoro-ethylene)					
Irradiated	0.03	0.45	0.12	0.01	0.01
Control	0.02	0.02	0.01	0.01	0.01
Difference	0.01	0.43	0.11	0.00	0.00

*Food Contacting Film = Polyvinyl chloride, 2 mil.

irradiation of the materials in contact with water produced no extractives. In contrast, the amount of the extractives decreased by 30 to 50 per cent after irradiation with 6.0 Mrads. When irradiated without contact with food simulating solvents, polyethylene is unaffected by radiation to an absorbed dose of 4.4 to 19 Mrads, depending on the kind of polyethylene.[5] Selection of a proper kind of polyethylene as a food contacting film seems to be of a great importance. As the data for the polyethylenes of the DuPont and 3M laminates indicate (Table 5), the polyethylene film used in the DuPont #15 series laminate was less resistant to radiation than the polyethylene specimen used in the 3M laminate when n-heptane was used as the food simulating solvent. The over-all extractives data for the 3M laminate (Table 5) indicate that this flexible packaging material is suitable for in-package radiation of foods from the viewpoint of the extractive formation on irradiation with 6.0 Mrads. However, the polyethylene used in the 3M laminate might be the source of the formation of undesirable gaseous products, as has been reported for various polyethylenes.[6,7] This possibility will be investigated. Irradiation of polyethylene with pasteurizing dose (1.0 Mrad) of the ionizing radiation, while in contact with distilled water as the food simulating solvent, produced measurable amounts of the extractives (Table 5). It appears that either irradiation with 6.0 Mrads improved the polyethylene films or the aluminum foil and the external layers of the laminates provided certain protection to the films during irradiation and storage. The second possibility is more

probable since polyethylene is subject to oxidation during irradiation,[5] and the exclusion of air by lamination with aluminum foil in the case of the DuPont and 3M laminates might have provided the necessary protection against the oxidation during irradiation.

Irradiation in contact with acetic acid produced measurable amounts of the extractives and their amount seems to be related to the radiation dose. Apparently, acetic acid dissolved certain non-polymeric components of the films since the data for chloroform soluble extractives indicate no change in the amount of the extractives before and after radiation (Table 5).

Extractives data for the Mylar-Foil-Vinyl laminate and the KEL-F film indicate that these films are also radiation resistant when irradiated in contact with water. Both films, like polyethylene samples, showed increased amounts of the extractives when irradiated in contact with acetic acid solution (Table 6).

In the case of the KEL-F film, the small amounts of the extractives formed by the irradiation when in contact with water and acetic acid solution might be fluoride compounds, as has been reported for another KEL-F film.[8]

KEL-F film showed the greatest resistance to the solubilizing effect of heptane as compared with other films. Polyvinyl film appears to be quite soluble in heptane, even without the effect of radiation (Table 6). It appears that for the plastic films soluble in n-heptane another fat-simulating solvent should be used as the film contacting solution.

Conclusions and Future Outlooks

The limited data on the extractives formation by 6.0 Mrad irradiation of polyethylene, polyvinyl chloride, and chlorotrifluoropolyethylene films, when in contact with water, indicate that these plastic films might be suitable for in-package radiation sterilization of neutral foods, such as meats.

The total amount of the extractives produced by irradiation is in the order of magnitude found for thermally treated polyethylene while in contact with distilled water.[9]

The absence of the chloroform soluble extractives in irradiated samples indicate that the dose of 6.0 Mrads didn't cause degradation of the polymers.

The FDA regulations[10] specify that the yield of net chloroform soluble extractives from packaging plastic materials shall not exceed 0.5 milligram per sq in. of the contact area. The four flexible materials investigated have met these requirements.

The research work, now being conducted by the Continental Can Company, will provide a more comprehensive picture on the extractive formation by various food packaging films. Should the result confirm the data

available for the four flexible materials, we can look with confidence for the in-package radiation sterilization of foods, at least from the point of view of their resistance to radiation and the clearance by the Food and Drug Administration for their intended use.

Realizing the type and severity of rough handling, which could be encountered in the military supply system, the development of acceptable flexible containers for the logistic purposes still will take a considerable length of time.

Looking ahead, we plan to have a few prototype flexible containers for radiation sterilized foods at the end of 1966; this will include the necessary clearance by the Food and Drug Administration from the standpoint of extractives.

Study of the performance of these prototype flexible containers with irradiated foods under different environmental conditions and during two years of storage at different temperatures will require additional time before final versions of flexible packages could be used for radiation sterilized foods.

We are optimistic, and our optimism combined with the needed support and "know-how" of the packaging industry will enable us, we believe, after four to five years of additional research and development work, to provide the U. S. Army, and eventually the U. S. Public, with wholesome irradiated foods packaged in flexible convenient packaging materials.

Acknowledgement

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SESSION NO. 5

Dr. J. Fred Oesterling, Chairman
U. S. Army Natick Laboratories
Natick, Massachusetts

DOSIMETRY AND DOSE DISTRIBUTION

Robert D. Jarrett, Sr.

In the time that has elapsed since the last Contractor's Meeting the dosimetry program has been concentrating on preparing and calibrating the radiation sources at the Natick Radiation Laboratory. To most effectively obtain this end, it was necessary to evaluate the available dosimetry systems and to make a selection of those which appeared to best meet the requirements of the food irradiation program. The requirements for dosimetry have been defined that it must be able to work under the following conditions:

1. Wide range of doses (10^4 to 10^7 rads).

2. Independent of energies of radiation.

   a. 0.6 to 2.2 mev for gamma.
   b. 0.6 to 24 mev for electron.

3. Wide range of dose rates.

   a. 10^5 to 10^7 rads/hour for gamma.
   b. 10^5 to 5×10^{10} rad/minutes for electrons.

While no single dosimeter meets the requirements, it is possible by using a family of dosimeters to fulfill the requirements. The dosimeter types should fulfill the following needs:

1. A "primary standard" dosimeter against which all other dosimeters could be checked and calibrated.

2. Secondary dosimeters, which would provide the requirements needed for operating conditions.

3. A simple, inexpensive dosimeter which could be affixed to each sample prior to irradiation so as to guarantee the sample has been exposed to radiation.

4. For the electron accelerator, a beam monitor that can measure the various beam parameters electronically and make adjustments in them so as to deliver a uniform surface dose.

In an effort to develop a dosimetry capability and to establish the dose variations in the sources at the U. S. Army Natick Laboratories, efforts were directed along several lines:

1. The problem was discussed with the NRC Committee.

2. Both an inhouse and a contracted literature search were conducted.

3. Capabilities in using proven dosimeter systems was developed.

The efforts produced a family of dosimetry systems which meet most of the above requirements, though not the ultimate. It is felt that with time and experience new and better systems will develop. I would like to discuss the systems which are now being used in this Laboratory.

The ferrous sulfate (Fricke Dosimeter) dosimeter, as recommended by the NRC Committee and the International Commission on Radiological Units,[1] is used as the primary standard by which all other dosimeters are calibrated. This system, with proper handling, is capable of an accuracy of ±1 per cent, but is greatly limited for use in this facility by its upper dose limit of approximately 50,000 rads. The dose rate in this facility is approximately 70,000 rads per minute, which means that the dose received in placing the dosimeter in the cell and removing it (transient dose) would be of the same order of magnitude greater than the dosimeters total range. This dosimeter has been used successfully for shielding studies and to calibrate other systems. For these studies locations were selected, in the cell, such that the dose rates were relatively low and the transient not a limiting factor.

The ceric sulfate dosimeter has been proposed by the NRC Committee and S. I. Taimuty[2] as the dosimeter to use for source calibration, despite its sensitivity to impurities. In reviewing the available literature, there appeared to be discrepancies with respect to its G values. Therefore, a research study was conducted with the assistance of Dr. Brynjolfsson on this system, the results of which will be published. These results may be summarized as follows:

1. By use of a simple calorimeter the G value (number of molecules converted per 100 ev of absorbed energy) of ceric sulfate was determined to be 2.32 ± .06 for all concentrations of ceric.

2. There is an increase in dose (D) received by the dosimeter due to the so called surface effect (ΔD) caused by the area (A) to volume (V) ratio of the ampule, which can be expressed as $\Delta D = 1.25\, D\frac{A}{V}$. (Fig. 1)

3. It is highly dependent on the spectral distribution of the gamma rays; as a result its response is dependent on the ceric concentration.

4. The G value is slightly temperature dependent (+0.17% per degree centigrade).

5. The G value is dependent on the cerrous ion concentration.

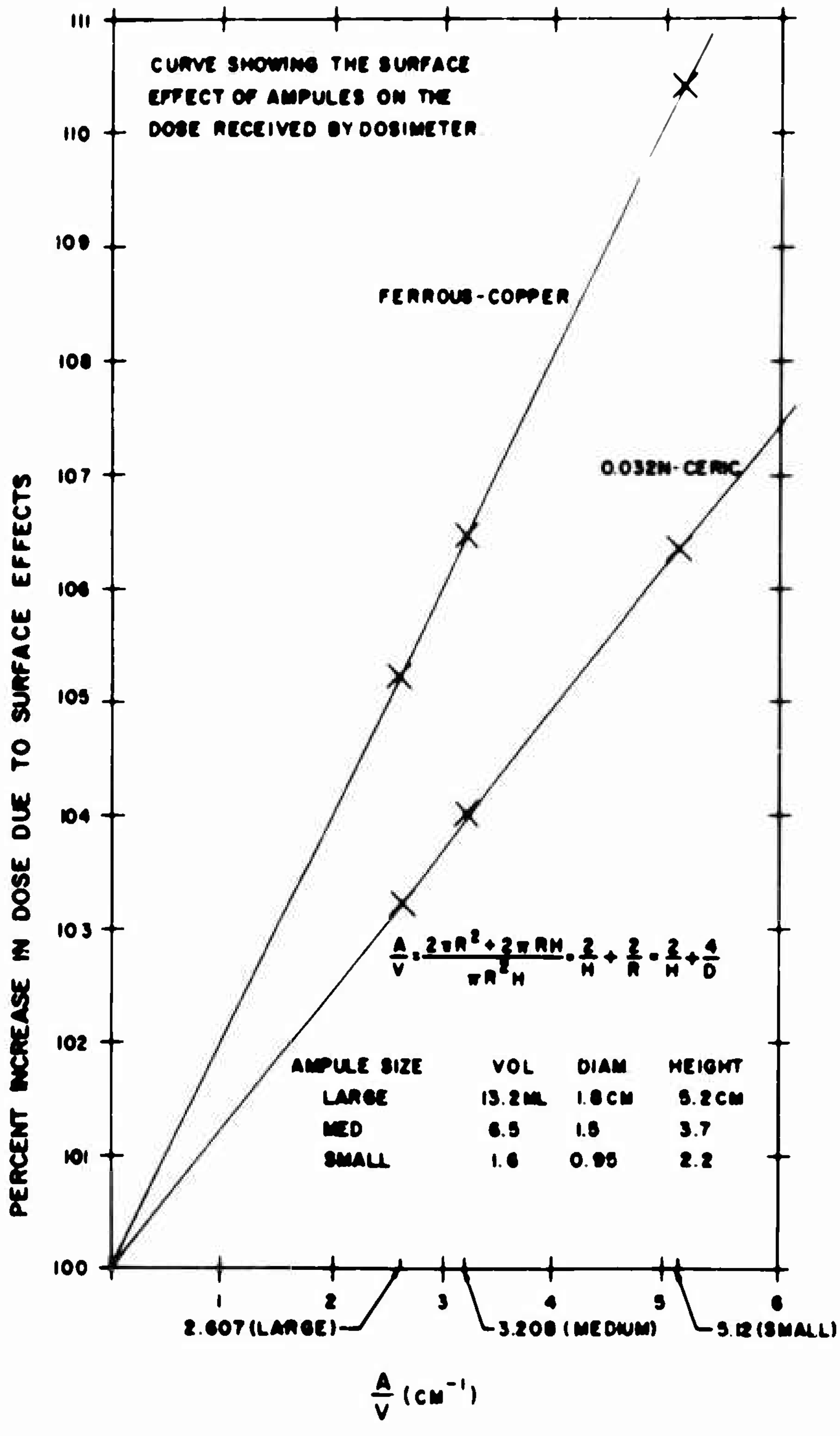

Figure 1. The increase in apparent dose received by the dosimeter as a function of the inner surface area of the ampule to the volume of solution.

Due to the fact that the ceric dosimeter is sensitive to the energy of the radiation (Fig. 2) and that the time of exposure necessary to obtain an accurate dose is relatively long, 30 to 60 minutes, it was felt that another system might be better. In my opinion, the modified Fricke dosimeter as proposed by E. Hart[3,4] would fulfill this need.

Ferrous-copper dosimeter has been used most extensively during the calibration of the cobalt source. It has been our experience that the increase

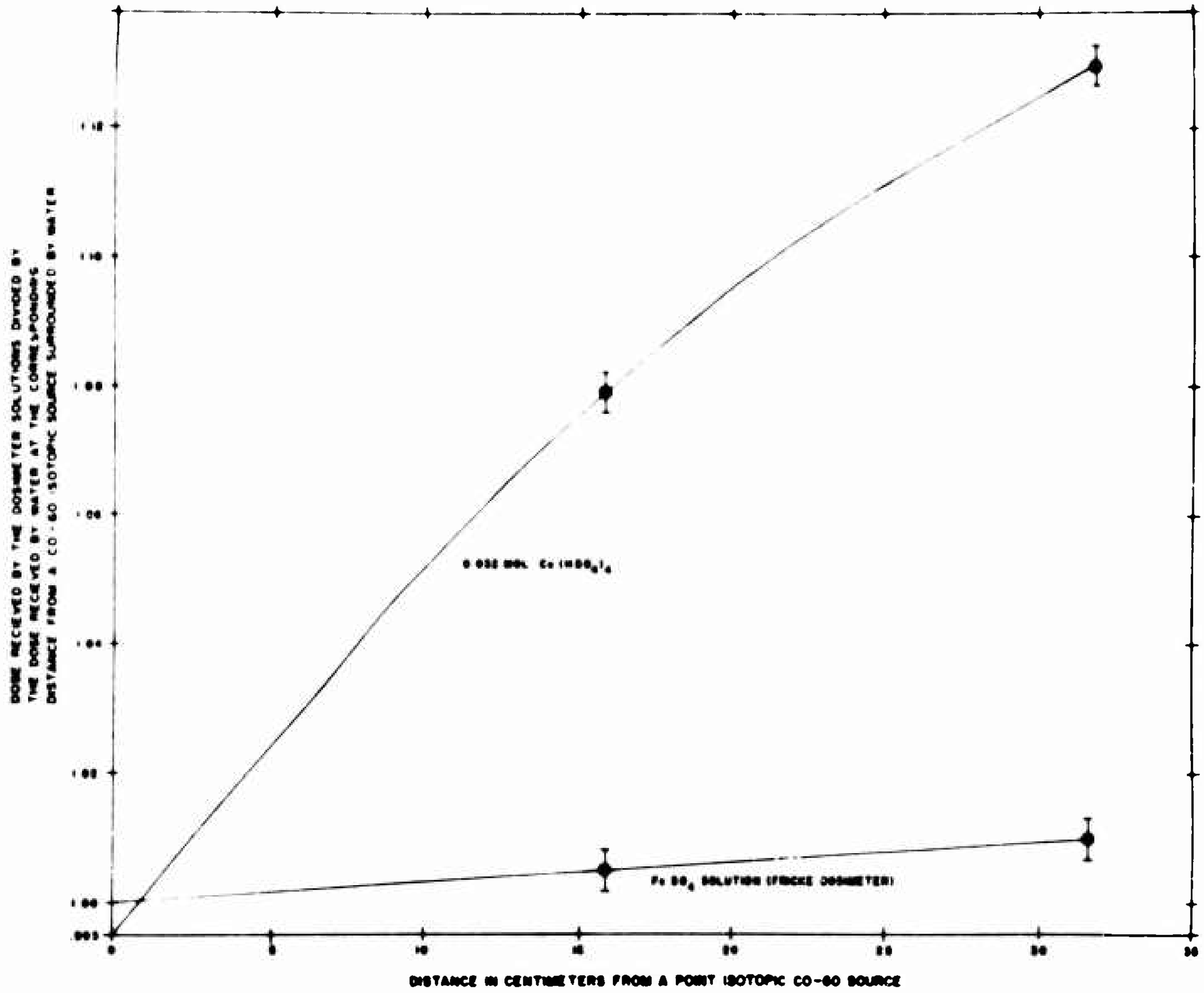

Figure 2. Increase in absorbed dose of ceric sulfate and ferrous sulfate as a function of the effective energy received from a point isotopic source in water.

in range of this system over the Fricke Dosimeter, 700,000 rads versus 50,000 rads, is advantageous. There are, nevertheless, the following problems with this system:

1. It is not stable, so it must be prepared the day on which it is to be used.

2. It is sensitive to the concentration of acid, so care must be taken when making the solution.

3. It must be irradiated in glass cells.

It has not been found that these problems appreciably affect the usefulness of the system. Although it is inconvenient to make the dosimetry solution fresh each day, the need for not calibrating each batch against a standard more than justifies its use. Since the opening of this Laboratory, we have been using

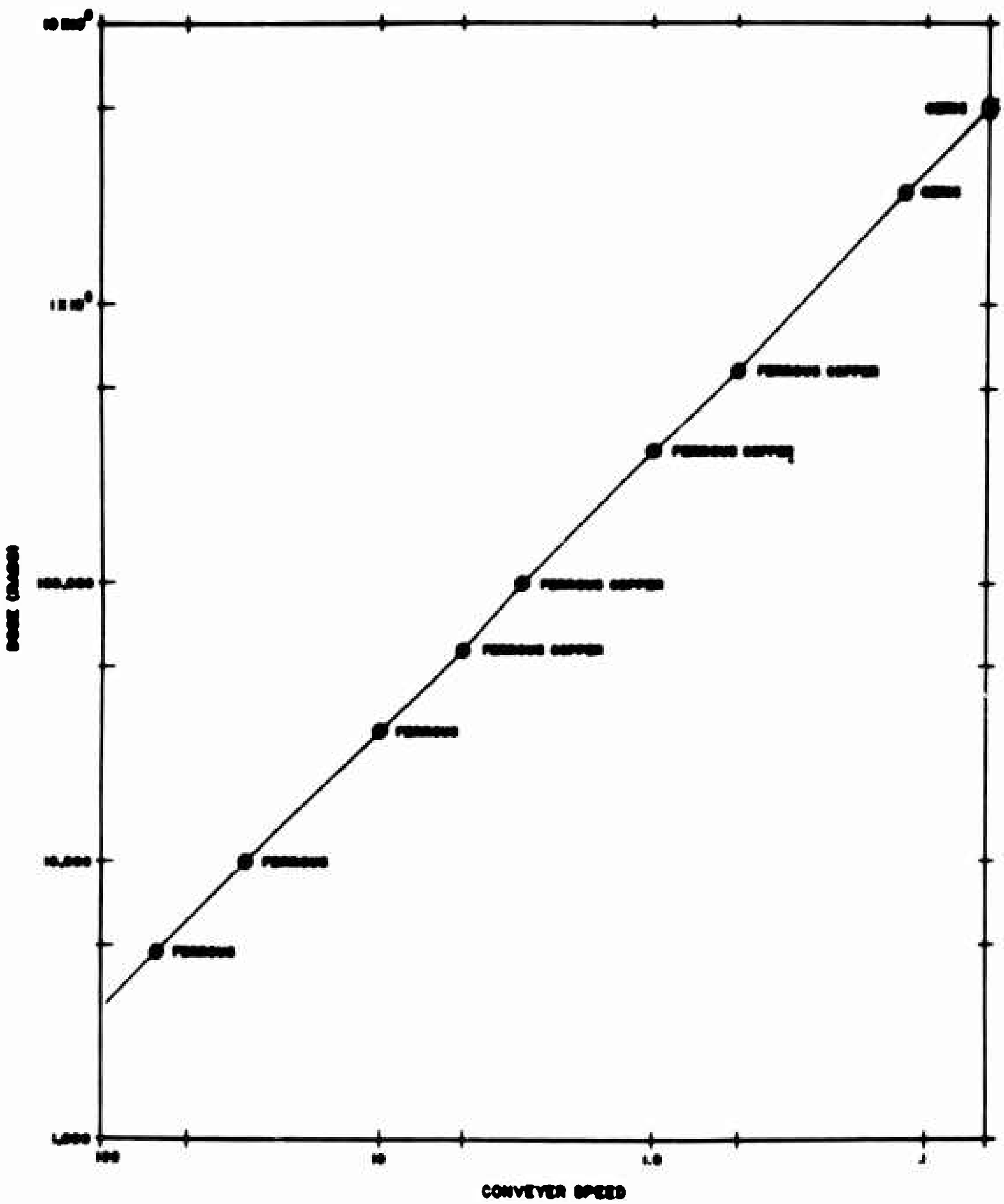

Figure 3. The dose values obtained with the use of ferrous-copper and ceric sulfate dosimeters are a continuation of the values obtained using the reference ferrous sulfate dosimeter.

ferrous-copper for routine dosimetry and have found it to be very reliable and accurate (±1%). Figure 3 shows that the values obtained using the ferrous-copper dosimeter are a continuation of the ferrous values thereby justifying its use. It is also relatively insensitive to the energy, therefore it is better suited for dose depth measurements than ceric sulfate. If there is a need for an in-line dosimeter, ferrous-copper cannot be used for the dose required to sterilize food is 4.5×10^6 rads. There has been development of an oxalic acid dosimeter in cooperation with the American Society for Testing Material (ASTM). There appears to be some problems in using this system. Mr. Holm, a visiting scientist from Denmark, will investigate the usefulness of this system in the food irradiation program.

The NRC Committee has suggested that there may be possible problems associated with dose uniformity, for example, in such areas as the surface between the can and food and the seam joints of the cans. In order to investigate these effects, it is necessary to have a dosimeter thin enough to see these fluctuations. The systems, in my opinion, that show the most promise are polyvinyl chloride (PVC) and blue cellophane.

PVC, as most systems, has its limitation, but it has been found that by standardizing on a procedure when using the system many variables are eliminated. We are using a commercial PVC (Bakelite VSA 3310, clear, 11 mils) and after irradiation are heating the film for 30 minutes at 80°C. This heat treatment causes the less stable trapped electrons to be freed and only the more strongly trapped to remain. It has been our experience that by calibrating the film, using a standardized procedure, the results are accurate to ± 10 per cent and the relative precision is good to ± 5 per cent. This system has been used successfuly in support of the package study being conducted by Continental Can Company.

In this study, sheets of PVC were placed between the film pouches which contained ferrous-copper dosimeters and irradiated. The results showed that the PVC can be used in this manner and the relative dose is in agreement with chemical dosimeters. The reason that we are at present using PVC in preference to blue cellophane is that it has more rigidity, which makes it easier to read in a modified spectrophotometer. Work will continue on PVC to improve our knowledge of its response to various electron energies and dose rates. It is planned to use this system for depth dose studies with the linear accelerator (linac). In addition, PVC has found use in determining beam location for the linac, and as an indicator that a sample has been irradiated. This indication is a result of the film changing from colorless to brown upon irradiation.

Hydrochloric acid is produced in the PVC film when irradiated. If a pH dye could be incorporated in the film, a more accurate go, no-go dosimeter would result. We have produced such a film which changes in color from yellow to red when irradiated. There needs, however, to be more research on this system as to the method of producing larger and more uniform batches.

Cobalt glass (Bausch & Lomb F-0621) has not been used to any great extent, but increasing use is foreseen. We have calibrated it for use in this facility and have investigated its stability.

While dosimeters are essential in determining the dose a sample receives, it is also necessary to place them in the food material to determine dose distribution. It has been found difficult to always use food for these studies so "phantoms" have been made of materials that have approximately the same energy absorption as food. It has been found that polystyrene and masonites are suitable materials for the construction of phantoms of which Figures 4 and 5 are typical examples. The phantoms are constructed so that they will hold the dosimeter in predetermined locations. When dosimeters are not used in all positions, they are exchanged with wooden dowels. By using phantoms it has been possible to map the source more rapidly than if foods had been used.

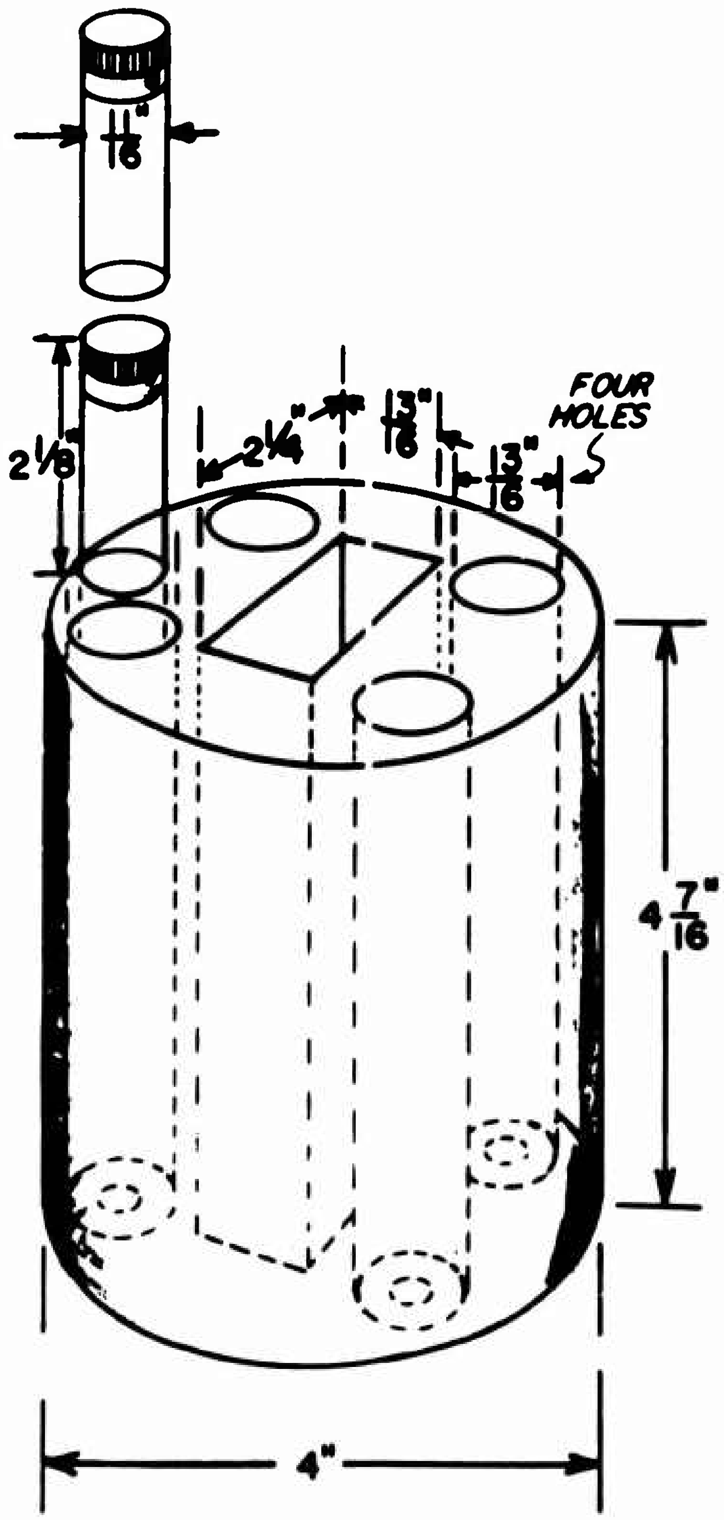

Figure 4. Typical dosimeter phantom made of masonite to simulate food with cell locations for dose distribution measurements in the cans.

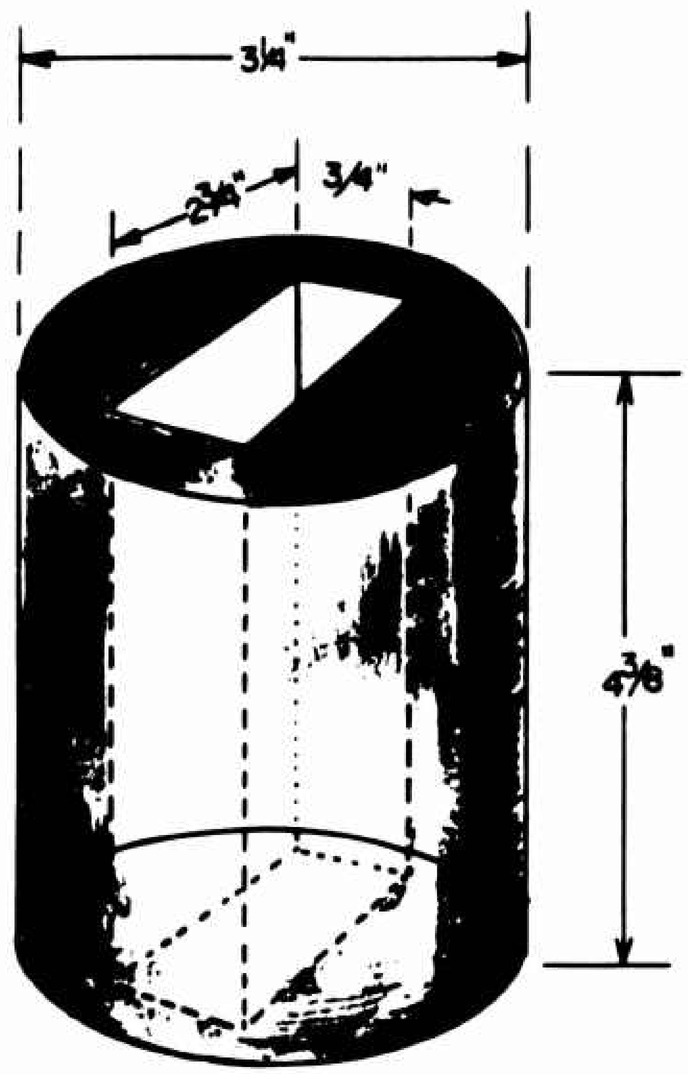

Figure 5. Polystyrene phantom used to monitor the dose uniformity in the irradiation carriers.

The results obtained using these phantoms showed that it was not possible to irradiate unit density material in cartons as it had been originally planned (Fig. 6), as the dose distribution was greater than 100 to 125 per cent. It was experimentally determined that if the cans were irradiated in standing position in the carriers (Fig. 7), with the addition of masonite

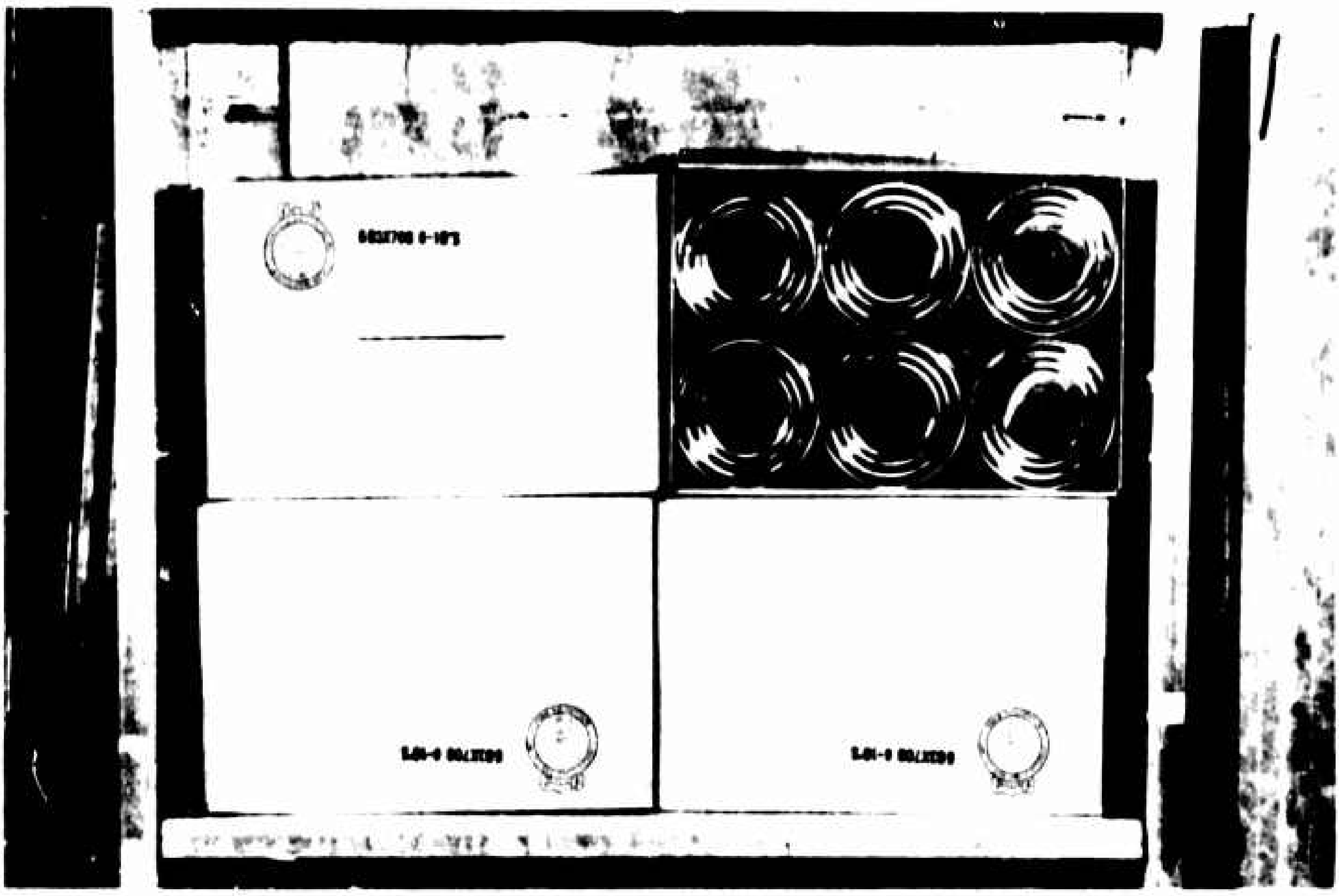

Figure 6. Package conveyor carrier loaded with cartons of No. 10 cans for irradiation. This method was replaced by that shown in Figure 7 because of dose non-uniformity.

absorbers, it is possible to obtain a dose distribution of 100 to 125 per cent (Fig. 8). The dose uniformity in a No. 10 can is approximately 100 to 107 per cent, with smaller size cans having a better dose uniformity.

As is evident, not much work has been conducted on the use of dosimetry systems with the linac. This is primarily a result of not having a suitable conveyor system. Work has, however, started on an electrical method of reading and controlling the beam parameters which, in effect, will provide a constant surface dose to the samples irradiated. Mr. Cooper will discuss this and other problems of the linac in more detail.

In addition to calibrating the source for food irradiation, does distribution and shielding studies have been performed on the cell and the labyrinth. The results are now in the process of being prepared for publication. These

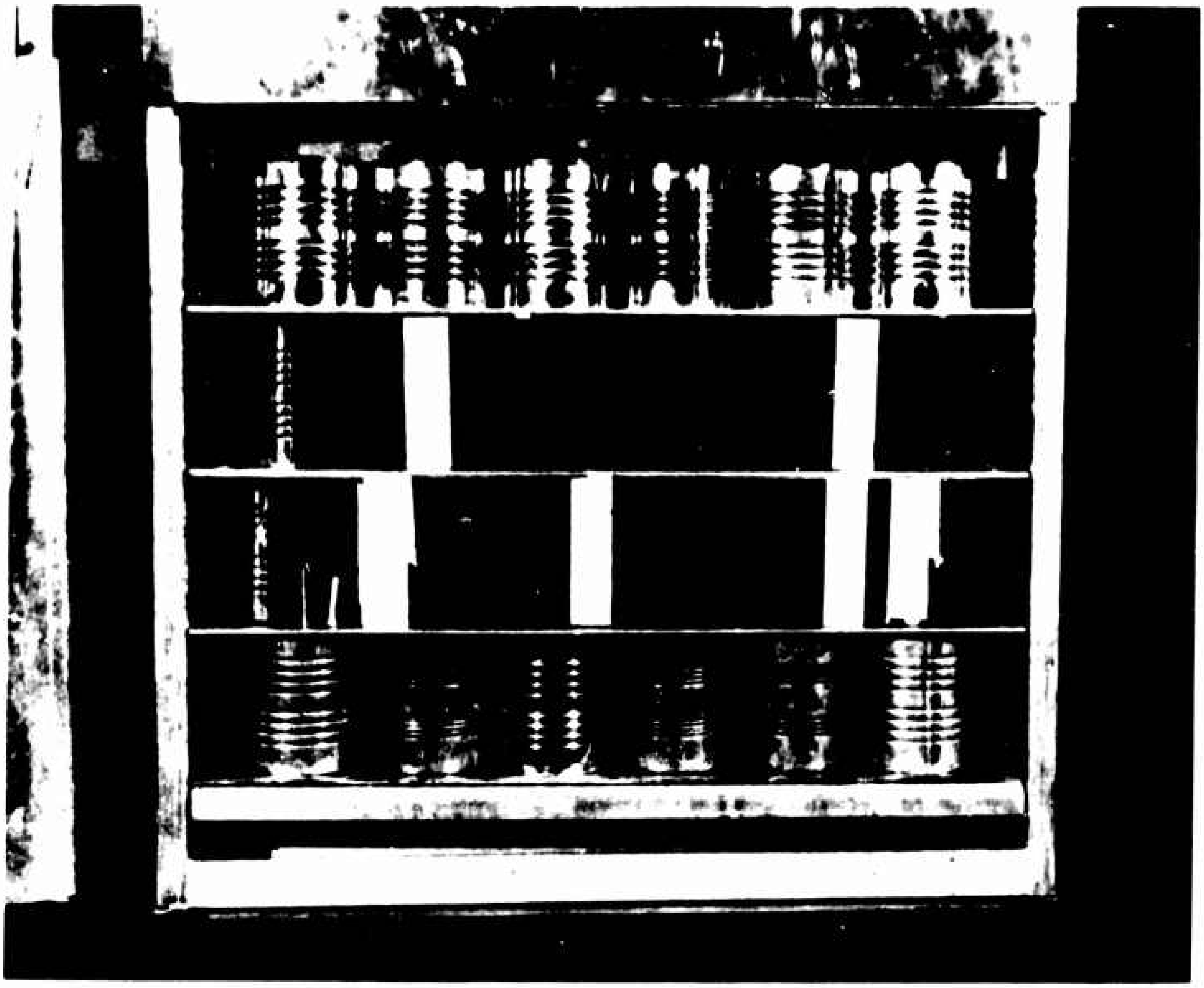

Figure 7. Cans located in the present irradiation configuration with masonite placed over the second and third row (from the top) to improve dose uniformity.

studies were conducted so that they might provide information for the design of future facilities. This facility is particularly suited for this study due to the large source of radiation and large labyrinth which facilitate accurate measurements.

Research will continue in the following areas:

1. Effects of changing concentration of reagents in making the dosimeters.

2. Calibration of polyvinyl chloride for use with the linear accelerator.

3. The effects of changing the irradiation energy and dose rates on the various dosimeters.

4. Develop and use electrical dosimeters such as ion chambers.

5. Investigate the depth dose distribution using polyvinyl chloride and blue cellophane.

6. Develop an inexpensive go, no-go dosimeter capable of indicating the dose received to ±5 per cent.

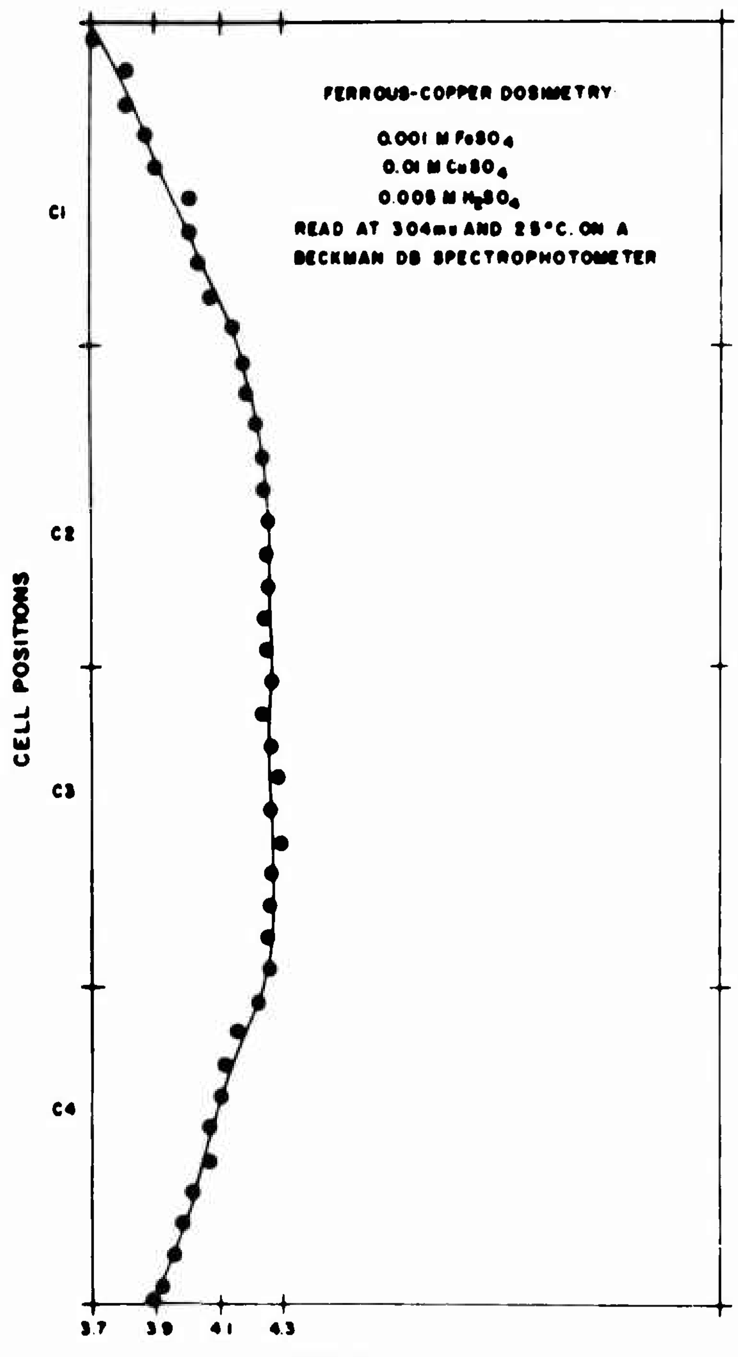

Figure 8. Typical calibration curve showing the dose uniformity in the center positions of four No. 10 cans phantoms from top to bottom in the third column of Figure 7.

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ACCELERATORS FOR FOOD PRESERVATION

Raymond D. Cooper

Introduction

I want to discuss the electron accelerator as a source of radiation for the preservation of food. Our interest will be particularly with linear accelerators, but, as we shall see, other types of accelerators might eventually be competitive in this field. These will be touched upon later.

In order to try to get a clear picture of the place of the electron accelerator in the food preservation program, the advantages and disadvantages associated with this application will be discussed in some detail. The advantages of electron accelerators for radiation applications have long been used by manufacturers as selling points, while the disadvantages and attendant problems determine the direction of our research effort. The research which has been accomplished in the year during which this Laboratory has been operational will be discussed together with the plans for future investigations. The objectives of this research program will be pointed out and tied to the problems and disadvantages of the accelerator. Finally, a brief attempt will be made to look several years into the future in order to determine the ideal characteristics of an electron accelerator system for the preservation of food. The economic aspects as well as the technical details will be touched upon in order to clarify the place of the accelerator in food preservation.

Advantages and Disadvantages of Electron Accelerators

Table 1 lists some of the advantages of an electron accelerator for use in radiation processing. This list applies particularly to linear accelerators. There are further advantages to simpler accelerators such as the Insulating Core Transformer and the Dynamatron of Radiation Dynamics.

The first advantage listed is not often considered. Dr. Brynjolfsson of the Danish Atomic Energy Commission discovered, while evaluating bids to furnish the Riso linac, that the costs of a large number of different machines could be represented by the formula given in 1(a). The \$50,000 term is just the cost of preparing the bid, engineering the accelerator, etc. The second term is the most important and indicates a cost of about \$20,000 per kilowatt of output power. This is basically just iron and copper needed to handle the power. There is a small term proportional to the energy in mev since the length of the accelerator and thus the amount of copper needed in the guides

TABLE 1

Advantages of Electron Accelerators

1. Relatively Low Initial Cost

   a. Linac cost = \$50,000 + \$20,000 x KW + \$4,000 x E + Accessories.

2. Efficiency of Operation

   a. Can be turned off when not in use.
   b. Electrons are utilized very efficiently.
   c. Great flexibility of beam utilization.

3. Relatively Low Operating Cost

   a. No decay as with isotope sources.
   b. Long klystron life possible.
   c. Lower prices of klystrons and diodes.

is proportional to energy. In addition, accessories such as a scanner and other special control and measuring equipment must be listed separately. The second major advantage is the inherent efficiency of utilization of the electron beam. To begin with, the accelerator need only be operated when samples are to be irradiated. This allows three shift operation if necessary or very infrequent operation if desirable. Second, the electrons are utilized well in the target because of the high specific ionization compared to gamma rays. The third point here is that the electron beam is very flexible and can irradiate either wide packages or narrow with equal efficiency. The final major advantage is the comparatively low operating cost since they do not decay as do isotope sources. Also, new, lower price, longer lived klystrons have been developed for the Stanford linac which will eventually lower operating costs on all accelerators.

Now that we have convinced ourselves that we should use an electron accelerator for food preservation, let us look at some of the disadvantages shown in Table 2. One of the most important problems is just the complexity of the equipment involved. This requires highly trained, highly paid personnel for operation and maintenance. Of course, as with any complex electronic device, there will be occasional breakdowns, and a certain amount of time will be required to set up the proper beam parameters. In addition, the accelerator must be shut down periodically for maintenance. The next major difficulty is the induced activity at higher energies. This requires that information be developed about the amount of activity in a particular food as a function of energy. Once a safe maximum operating energy has been defined, it will be necessary to control the energy carefully so that the accelerator will operate at this energy but no higher. Since we will be limited in energy, the

TABLE 2

Disadvantages of Electron Accelerators

1. Complexity of Equipment

    a. Requires highly trained operating and maintenance personnel.
    b. Occasional interruptions in operation.
    c. Longer setup time required.

2. Induced Activity at High Energies

    a. Requires careful energy control.

3. Low Penetration

    a. Total range is 0.5 cm per mev for unit density material.
    b. Cross firing required.

        i. This implies control of energy and sample density.

penetration of the electrons will be limited. It is therefore necessary to make maximum utilization of the energy which can be used. One technique which has been applied to thicker samples is cross-firing or irradiating from both sides. If this can be made to work, it will more than double the thickness of sample which can be irradiated. However, because of the large slope of the forward edge of the dose distribution curve, the dose in the center resulting from the addition of electrons from both sides is very dependent upon the energy and the density of the target. A small variation in either of these will result in a very large variation in dose.

The disadvantages listed above determine our research program to a great extent since we wish to minimize or solve these problems. Thus our first research objective has been to make the accelerator more reliable, more stable, and more easily operable. Towards this end, a number of modifications have been made in the accelerator and its controls. One of the first improvements made was in the gun which injects electrons into the accelerator guide. Previously, the injection current had varied by as much as 5 per cent during even relatively short runs. By the addition of a difference amplifier and feedback, the long term drift has been reduced to the order of one per cent. The high voltage circuitry has also been improved so that the klystron output and thus the energy of the electrons will be held constant. An energy analyzer shown in Figure 1 has been added to the accelerator. This makes it possible to read the energy out in mev from a digital voltmeter or to plot in a very short time the distribution in energy of the beam. The drive and power supply system for the scanner has been rebuilt in order to improve reliability and decrease the drift. The scanner controls have also ben consolidated to ease the operator's job.

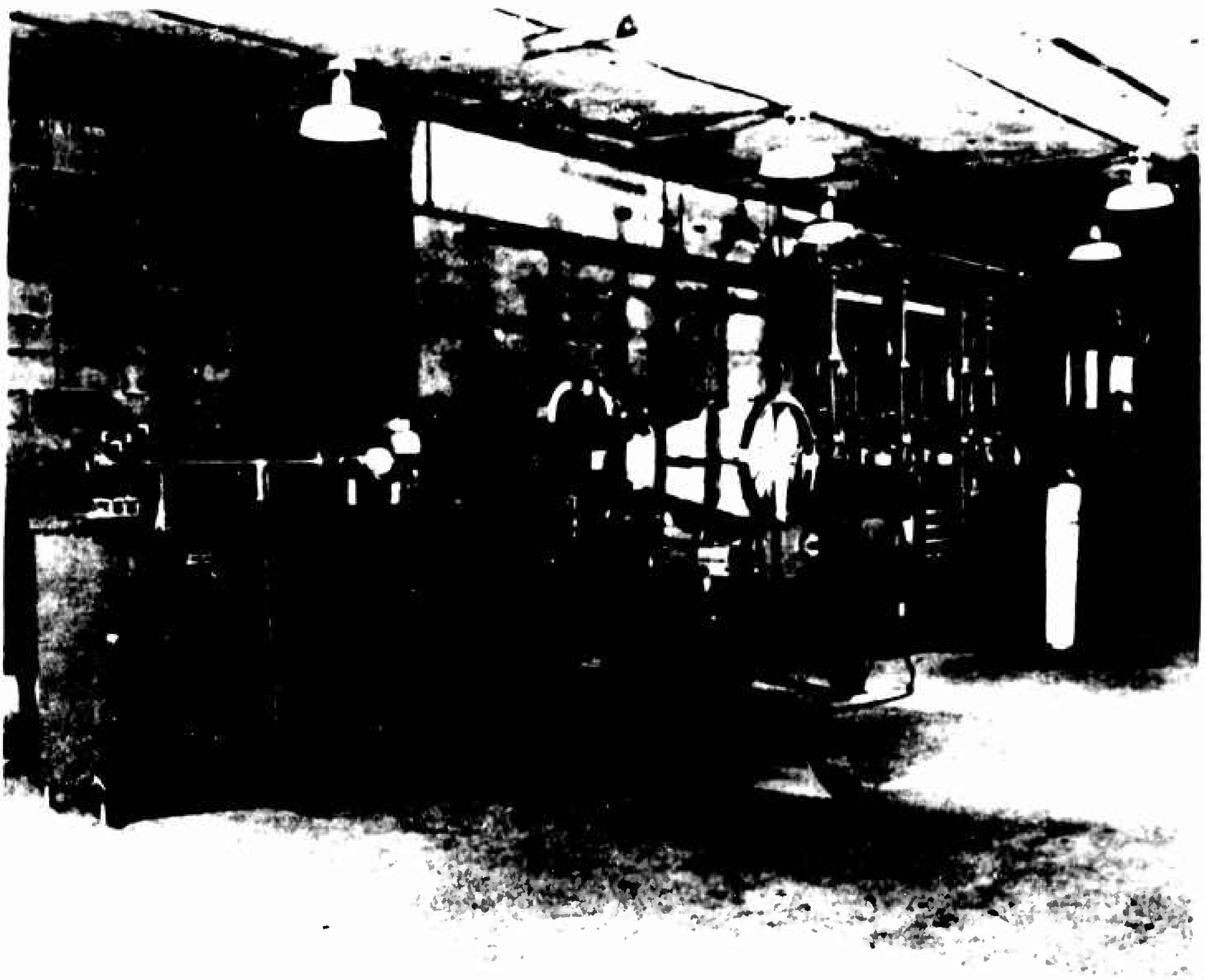

Figure 1. Electron Linear Accelerator with Scanner and Energy Analyzer.

The conveyor system for the accelerator is being built and will be installed before 20 December 1963. This has been designed so that the speed will be very accurately controlled in the region of the scanned beam. The beam current will be measured continuously by secondary emission monitors built into the scanner. The signal from these monitors proportional to current will be used to control the speed of the conveyor. Thus if the current were to drift down, the conveyor speed would be automatically lowered to keep the dose constant.

In order to help the operator set up and maintain a certain dose to the samples, a device has been designed and will be built which will indicate the surface dose continuously on a digital voltmeter. It will do this by measuring the parameters which determine the dose such as current, conveyor speed, and scan width and calculating the resultant dose. A multichannel recorder is also being purchased so that a record can be made of all the important beam parameters and the calculated surface dose.

A start has been made during the past year on studying the second major problem, induced radioactivity in food due to high energy electrons. An extensive literature search has been made to gather all available information on photonuclear cross-sections, thresholds, isotopic masses, and decay schemes. This has been written up in the form of a handbook and will be reproduced in the near future. Experimental studies have also been started to determine the amount and kind of radioactivity produced in beef by electrons of several different energies. Ashed beef samples have been exposed to very high doses from the linear accelerator at well known and controlled energies. Quantitative measurements were then made to determine the radioactive isotopes present. This work is being continued, and the results will be compared to calculations of induced activity made from the known cross-sections and isotopic abundances in food. Later studies will be made of the distribution of activity as a function of depth in the sample.

The final major problem was concerned with obtaining a uniform dose throughout the sample. Since this requires knowledge of dose distribution, backscattering, and many other factors, a research program has been started to obtain a complete understanding of the interaction of electrons with materials. The first experiment in this program, a study of charge distribution in water, oil, and aluminum, has been completed. Figure 2 shows a drawing of the apparatus used to make these measurements in liquids. The energy analyzed beam passes through several apertures and into the liquid through a thin aluminum window. The charge is collected on an insulated aluminum plate which is moved through the liquid during irradiation. The experimental results for the charge distribution in water normalized to the same practical range are shown in Figure 3. The curves are seen to be gaussian in shape with a low energy tail. As would be expected, the higher energy charge distributions are narrower and more nearly gaussian. Near the front surface of the sample, the electron beam knocks more charge out than is stopped, resulting in a net positive charge. This causes the charge distribution to start below zero at the front surface. The charge removed from this region adds to the low energy tail. Figure 4 shows the integral of the charge distribution curves for oil. These can be considered transmission curves. Note that for high energies just below the surface there are more electrons passing through than there are in the beam. This charge distribution study has been completed and is being prepared for publication.

Studies similar to those described above are planned of dose distributions. Although considerable information exists on dose distributions below 15 mev, very little data is available at higher energies. This work will include a thorough investigation of the cross-firing technique. In addition, studies will be made of backscattering as a function of energy and material. Later investigations will cover angular and energy distributions of the backscattered electrons in order to obtain a complete picture of the interactions. The final step will be to try to calculate dose and charge distributions in order to compare with the experimental results.

It is clear that the final characteristics of the accelerator system for food irradiation will be determined by the results of the investigations now

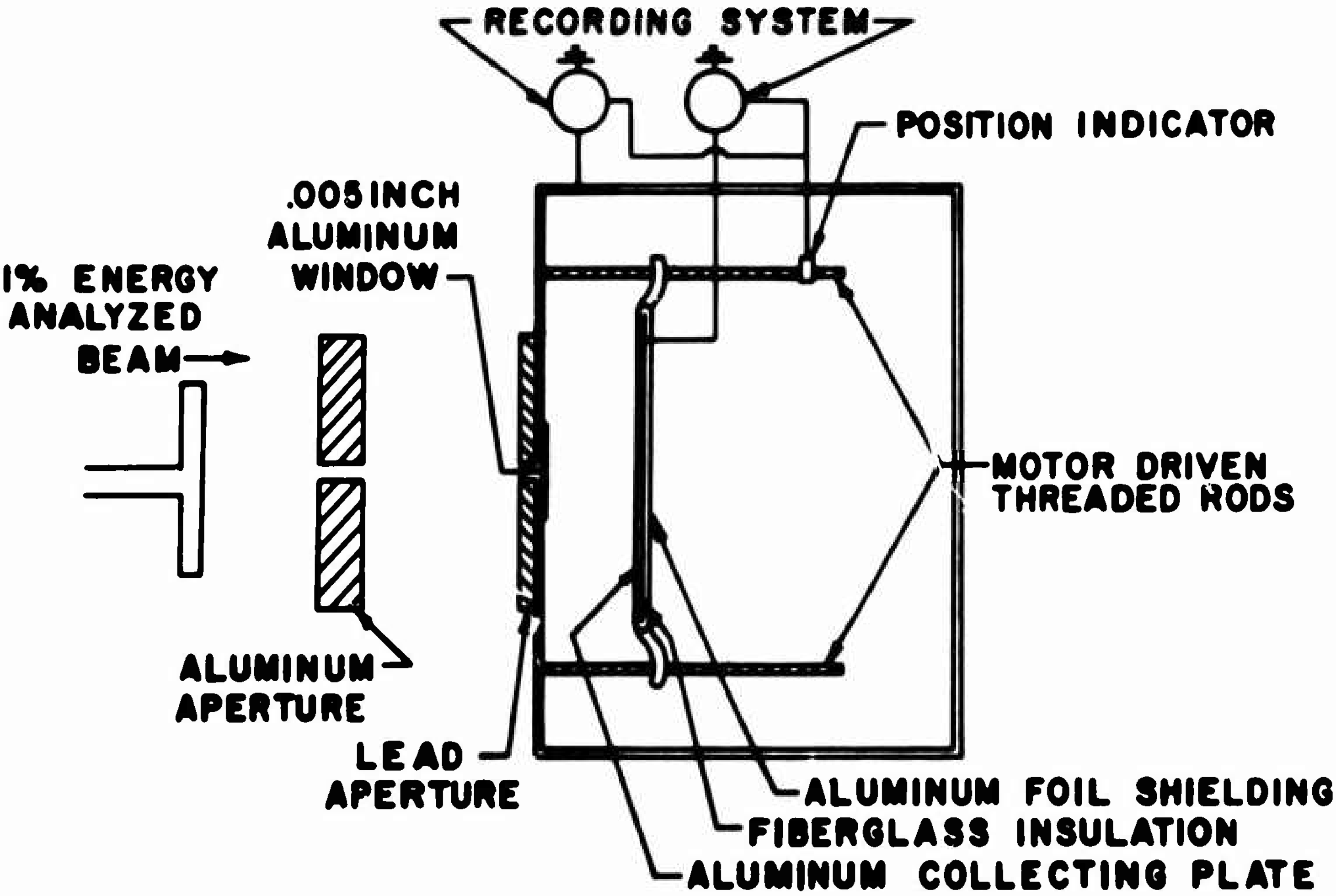

Figure 2. Apparatus ıor Measuring Charge Distributions in Liquids.

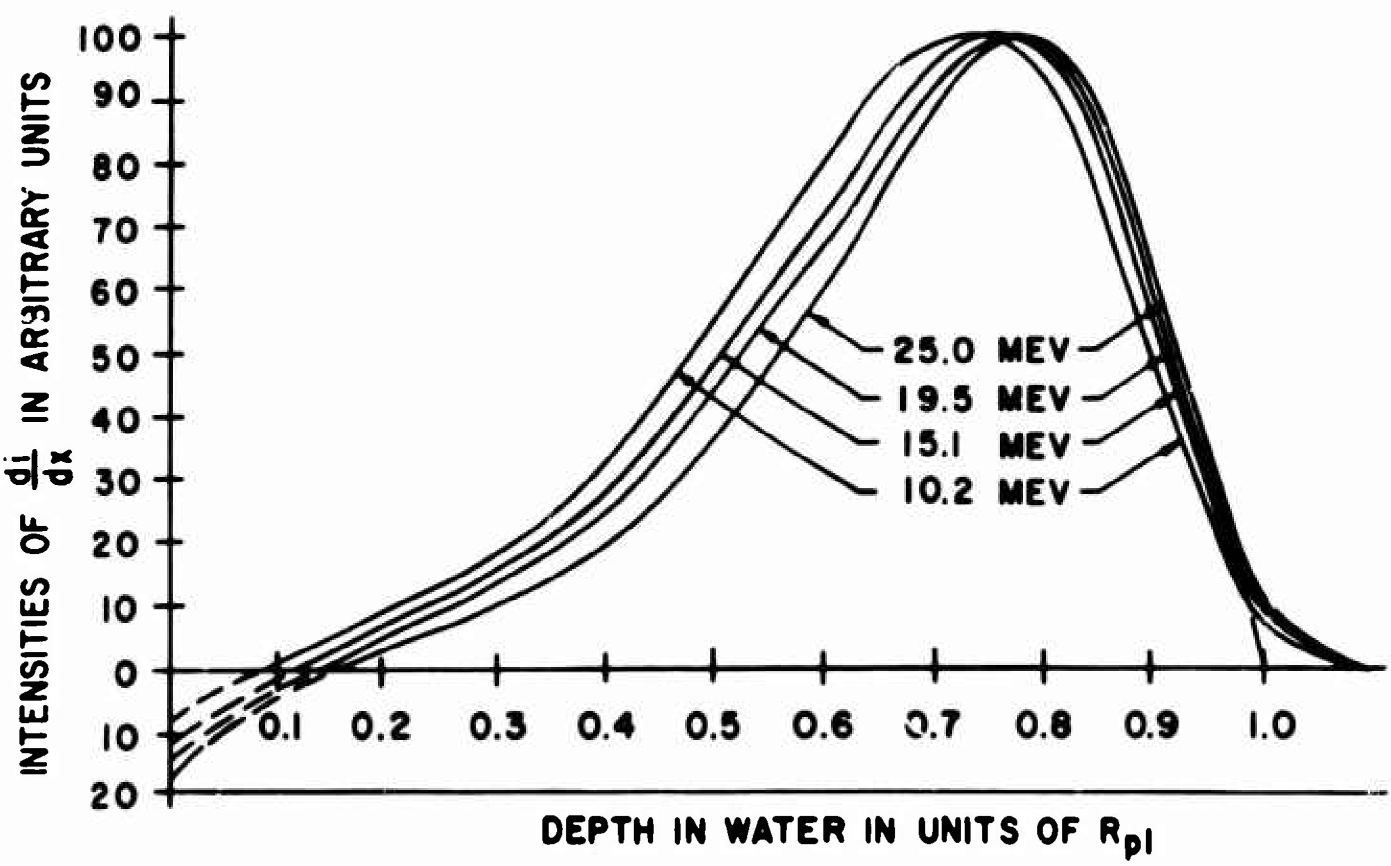

Figure 3. Charge distribution $\frac{di}{dx}$ in water as a function of depth.

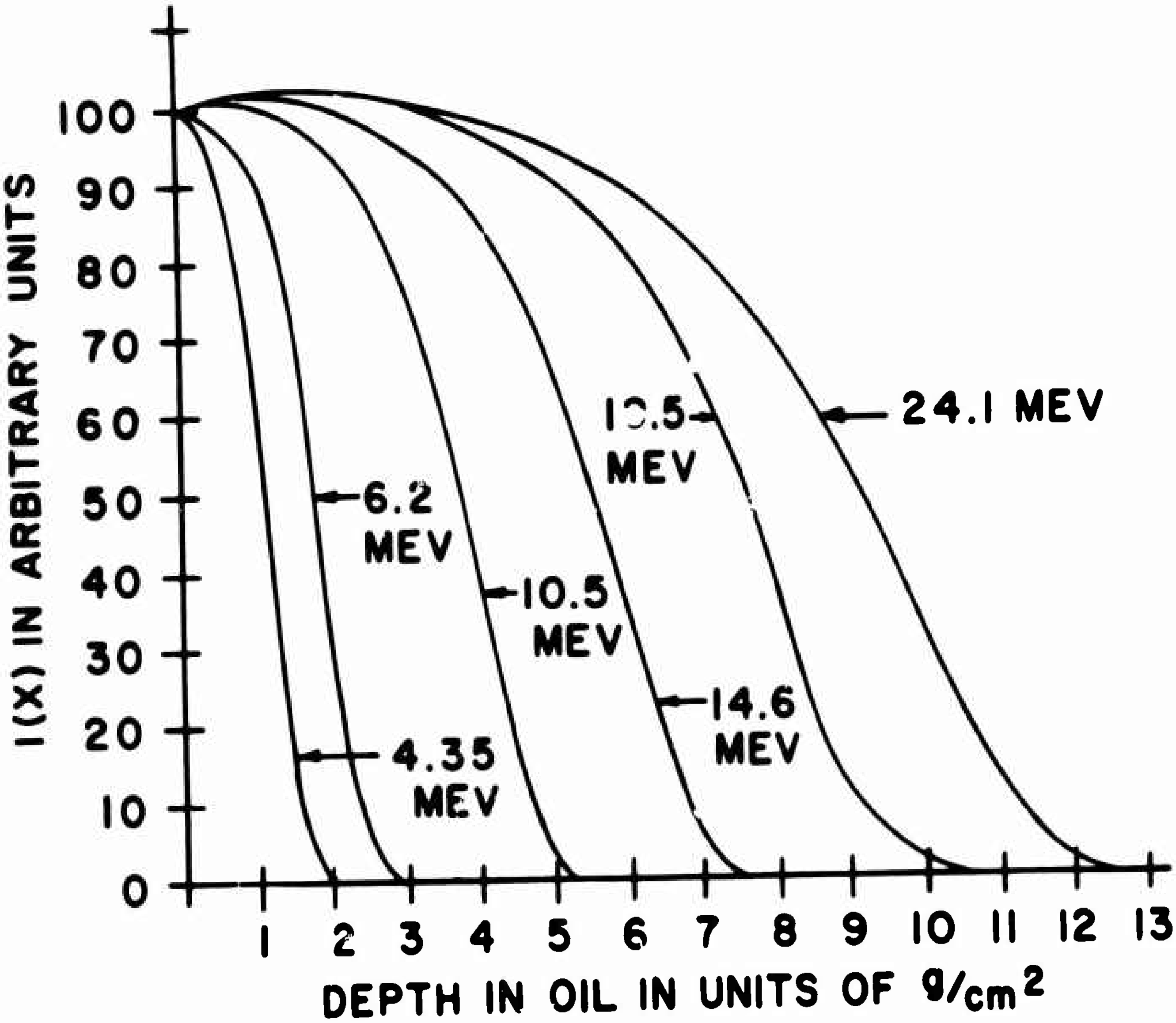

Figure 4. Charge transmission I (X) in oil as a function of depth.

under way. For example, the accelerator energy will be determined by the results of the induced activity studies, and this in turn together with the cross-firing technique will limit the maximum size of the package which can be irradiated. With the large advances which have been made in accelerator technology during the last few years however, and the continual decrease in cost per kilowatt, there can be no doubt whatsoever that high energy electrons will play an increasingly larger part in the radiation preservation of food.

CONFERENCE SUMMATION

Edward S. Josephson, Narrator

The AEC Program

Product Development (Kevin G. Shea)

Significant progress has been made during the past year toward the development of technology necessary to extend the radiation pasteurization of several fish and fruits to the point of semi-commercial processing. In several cases, the process outlines have been determined; in others, it is imminent. A study to determine extractables in packaging of irradiated products is nearing completion, to be followed by appropriate requests for FDA clearance of these packaging materials. Conceptual designs for a wheat irradiator and an on-ship fish irradiator have been completed. Long term animal feeding studies have been initiated on soft shell clams; similar studies on two fruits are planned.

Large scale irradiation of several fruits and vegetables, utilizing a mobile cobalt-60 irradiation unit is taking place in the California area. This program will allow the treatment of sufficient produce to conduct large scale storage and shipment tests, to help determine more exactly the economics of radiation processing, and to demonstrate on a wider scale the feasibility of the process.

Construction of the Marine Products Development Irradiator, located in Gloucester, Massachusetts, is proceeding on schedule. When completed in late summer, 1964, this facility will have a capacity adequate for conducting large scale radiation pasteurization technology of sea foods, and to acquaint industry with a prototype irradiation facility.

Industry interest in food irradiation has increased notably with the FDA clearance of irradiated bacon in February, 1963. This petition was submitted by the Army. The FDA has also approved the clearances of irradiated wheat and wheat products and of the use of 5 mev electrons for sterilizing bacon. Petitions have been submitted for low dose treated citrus products and packaging material for use with irradiated foods. Other petitions planned during the coming year include one or more fruits and possibly one or more sea foods.

Wholesomeness (Leo Whitehair)

The Commission's Division of Biology and Medicine supports studies in the areas of wholesomeness, microbiology, biochemistry, and physiology of low dose irradiated foods.

Two year animal feeding studies on soft shell clams and protein availability studies on haddock, flounder, and crab have recently been initiated (University of Illinois). The protein availability data will essentially complete the required wholesomeness data for haddock, crab, and flounder. Long term animal feeding studies on strawberries will also be conducted (Industrial Bio-Test Laboratories, Inc.). Foods for these wholesomeness studies are being irradiated at the Natick facility.

Two studies on growth and toxin production of various strains of *Cl. botulinum* Type E in various substrates and media, as well as marine homogenates, are in progress (University of Michigan and Continental Can Co., Inc.). The ultimate objective of both of these studies is to evaluate any possible public health hazards involved in the prolongation of refrigerated storage life of food by application of low doses of radiation.

Microbiological and biochemical studies on irradiated fish are under way at the University of Washington. Efforts have been concentrated on the bacteriological safety of radiation pasteurized sea foods. Potentially pathogenic organisms are being studied to determine their sensitivity to radiation, outgrowth after irradiation, and physiological changes after irradiation. Biochemical studies are designed to identify products and mechanisms involved in the tissue changes and relate them to radiation dose levels.

Basic studies on the radiation biochemistry of fruits are being conducted at the University of California (Davis). The effects of ionizing radiation on cellular and intracellular structure and metabolism of fruit tissues are being explored. Investigations on the physiology and microbiology of fruits are also being conducted.

The biochemical and physiological changes in fresh fruits and vegetable tissue (associated with extension of shelf life by means of radiation) are being studied at Cornell University (Geneva Agr. Expt. Sta.). Softening changes which occur in plant tissue during and after exposure to radiation will be investigated.

Fundamental flavor and odor studies on volatiles from irradiated fish are being conducted at the Gloucester Laboratory. Attempts will be made to correlate gas chromatogram patterns with detailed results of organoleptic testing on the same fish products.

A study on the biochemical alterations in the protein moiety of hemoprotiens (fish myoglobin) as a result of radiation is being made at the University of California (Berkeley).

The findings in each of these studies will be welded with previous results obtained in the Army's program and with results of studies currently supported by the Division of Isotopes Development. We are confident that these combined efforts will culminate in the successful use of radiation as a means of extending the storage life of perishable foods.

Development of Radiation Facilities (George R. Dietz)

A major portion of the AEC program on low dose food irradiation has been devoted to the development of a family of radiation facilities. Initial emphasis was placed on installation of research irradiators at various research sites with the prime objective of providing immediate irradiation support to food program contractors.

With this phase of development nearing completion, full attention has now been turned to semi-commercial or pilot plant facilities designed to demonstrate radiation feasibility, to prove out on a near-commercial scale the favorable results of laboratory tests, and to more realistically determine the economics of radiation processing. The Marine Products Development Irradiator, scheduled for completion in late summer, 1964, will fulfill these objectives with respect to sea foods, while a transportable or mobile cobalt-60 unit will be constructed by early 1965 for use with fresh fruits.

Other designs which have been made include those for a grain irradiator and an on-board ship irradiator. The former is planned for construction this fiscal year (FY 1964), while the further development of the latter is expected to depend on the successful results of the MPDI and the related interest of the fisheries industry.

In addition to cobalt-60 as an approved food irradiation source, the U. S. Food and Drug Administration has approved the use of electrons at 5 mev energy, and any sealed gamma ray source of less than 2.2 mev energy. None of the above impart radioactivity or radiation to the food.

Engineering interest, know-how, and capabilities are sufficiently developed to assure that appropriate facilities can and will be built when the food industry and the consuming public are ready to utilize radiation processing.

Economic Aspects (Joseph E. Machurek)

Marketing and economic studies of low dose radiation processed sea foods and fruit products indicate that:

1. Expansion of fresh fish markets and maintenance of quality are the prime benefits expected of the radiation process. A radiation cost of 1¢ to 3¢ per pound for these products could be tolerated by about 60 per cent of the fish industry, while a cost of 1¢ per pound or less

could be tolerated by over 90 per cent. The former cost is reasonable to expect during early commercialization, while the latter could be attained as facility development progresses.

2. The economics of fruit irradiation are less favorable than for fish. Where chemicals are presently used and perform in a satisfactory manner, economics of fruit irradiation appear to offer no clear-cut advantage. Where chemical treatment is inadequate, such as for post-harvest storage of strawberries, irradiation does offer excellent results at an economically competitive cost.

3. Several petitions are currently either in the hands of FDA for clearance of low dose products or planned for submission in the next few months. With the clearance of a "base" of irradiated foods, it is felt that true commercialization could begin in the next two to three years.

4. Isolated commercial uses, such as for potatoes, could begin within the next year or two.

Radiation cannot be highlighted as a food preservation cure-all, but there is every reason to believe it can stand on its economic and preservation merits with a fair number of various products. For these reasons, we continue to be impressed and enthusiastic about its ultimate commercial success.

The Army Program

Wholesomeness (Nicholas Raica)

Before the food radiation preservation program could be appropriately launched, it was realized that research had to be conducted to determine if the degradated fats and proteins caused by ionization radiation possessed any toxic properties that would ultimately lead to an unwholesome product. It was also recognized that foods incompletely processed are subject to spoilage mechanisms such as microbial, enzymatic, and oxidative, and could thereby result in unwholesome food products.

Simultaneous studies began nearly ten years ago on the microbiology, radiation effects, and acceptability of irradiated food by the Army Quartermaster. The Army Medical Service shortly thereafter implemented a wholesomeness program of such an unprecedented magnitude that involved feeding experiments with tens of thousands of animals over several generations and short-term feeding studies with human test subjects. Assays were made for such results as vitamin loss and residual enzyme activity. Histopathological studies were performed to determine if the feeding of irradiated foods caused any tissue changes in experimental animals.

From some of the early reports that poured in, it became apparent that the Food Radiation Program was in jeopardy and may be destined to a very

short life. The anomalies that were observed in long-term feeding tests were at first considered to be caused by toxic substances in the food irradiated at a dose of 2.79 to 5.58 Mrads. However, later analysis and repetition of tests of irradiated food showed that the anomalies were not manifestations of toxic compounds but were caused by such things as nutritional inadequacies in the total diet, too high a level of the challenge food for the normal consumption of the animals, or by factors independent of the irradiation of the food. Thus far, there has been no evidence presented to indicate that irradiated food is toxic or carcinogenic. However, there are a few remaining doubtful areas that need resolving, but these are being explained satisfactorily by studies currently in progress. The destructive effect that radiation has on vitamins is variable and differs from food to food and conditions at the time of irradiation. In general, the effects are equal to that of heat processing and offer no special problems.

It is felt that the wholesomeness of foods irradiated within the limits set in 1953 has been adequately demonstrated after ten years of intensive study. Any further work should be evaluated on the basis of experience and knowledge gained from these and other related studies. The use of higher energy electron beams seems to offer many technical advantages; however, with the use of such sources, new criteria for the establishment of wholesomeness may be required.

Studies have shown that there is no detectable induced radioactivity in foods irradiated with 10 mev electron beams. Calculations, based on four food items to simulate the total diet and considering the isotopes Na^{22}, I^{126}, Zn^{65}, Fe^{55}, and Mn^{54}, indicate that the body burden by ingesting 24 mev irradiated food would be 0.26 mr/year. This quantity appears insignificant when compared to the body burden and external irradiation from natural sources, which have been estimated to be about 150 mr/year of which 5 mr is contributed by fallout products.

Product Development (Fred Heiligman)

Research in the Product Development Program is divided into three areas:

1. Pre-irradiation, where studies are being done to determine the influence of variations of factors that can be controlled prior to irradiation.

2. Concurrent-irradiation, which is involved with factors that can be controlled while the produce is being irradiated.

3. Post-irradiation, which deals with factors involved in the utilization of irradiated products.

In the pre-irradiation area, such factors as variation in type and quality of raw materials, methods and extent of enzyme inactivation, the use of selected

additives and odor and oxygen scavengers, and packaging environment have been shown to influence the acceptability and storage stability of the products.

Enzyme inactivation by methods that require the shortest periods of time usually yield the best irradiated products. The use of low grade beef results in a better irradiated beef product than the use of high grade beef. This seems to be related to the tenderizing effect of both heat-enzyme inactivation and radiation on connective tissue. The amount of total and intramuscular fat in raw pork loins has some affect on the acceptability of irradiated roast pork loins, particularly when the product is served cold. The variations in curing methods used by different commercial companies in preparing such items as ham and bacon seem to markedly influence the acceptability of the irradiated products made from them. The causes of this are not known but are being investigated. The use of activated charcoal in the head space of packaged irradiated foods does absorb odor and probably improves the acceptability of the irradiated products. Vacuum packaging seems to be required in order to retain qualtiy in the irradiated products.

In concurrent irradiation area, research is being done to determine the influence of total dose, dose rate, and product temperature during irradiation on product quality. In both hams and bacon, reduction of the dose from 4.5 Mrad to 2.5 Mrad seems to markedly improve product quality and storage stability.

In all experiments in which the product temperature during irradiation was kept below freezing, the quality of the irradiated products seems to be improved. This has been observed in beef, chicken, turkey, and hams. Radiation flavor intensity seems to be dependent upon product temperature during irradiation and the relationships appear to be linear.

In the post-irradiation area, many recipes and preparation procedures have been developed for utilizing irradiated meats. It was pointed out that standard culinary practices frequently require alteration when irradiated meats are used. This is due to cooking during enzyme inactivation and changes in sensory characteristics associated with irradiation and storage.

The results of several acceptance studies on different types of irradiated meats (beef steaks, bacon, pork roast, pork chops, chicken, and barbecue pork) were presented. These particular studies were selected because they presented results that show irradiated food can be produced that has good acceptability characteristics and will remain stable in storage. Problems of reproducibility of results were discussed.

Biochemical Aspects (A. S. Henick)

The pioneering studies supported by the Radiation Preservation of Foods Program on chemical changes in food components resulting from ionizing radiation were reviewed. Most of the reactions in aqueous systems were

indirect effects involving interactions with the ions and free radicals produced in water. In dry systems, direct effects yielded radicals and ions, which in some cases reacted in the water or oxygen subsequently introduced.

The principal reactions in carbohydrates were hydrolysis of glycosides, followed by oxidation of monosaccharides. Amino acids were deaminated, decarboxylated, oxidized and/or reduced in proportions depending upon the presence or absence of oxygen. Sulfur in the molecule increased its radiosensitivity, giving rise to reactions which are related to flavor changes in protein foods. Aromatic and heterocyclic rings in amino acids were also affected.

Denaturation, fragmentation, and polymerization were reported in irradiated proteins, resulting in changes in solubility and viscosity of their solutions. The capacity of the proteins to bind dyes was also altered. Native lipoprotein was reported to protect other proteins in food systems. Protein peroxides were also reported reaction products of irradiation in the presence of oxygen.

Fats and fatty acids, both saturated and unsaturated, were oxidized during irradiation. In part through destruction of antioxidants, but also apparently through stable free radicals, susceptibility to post-irradiation autooxidation was increased in irradiated lipids. Reaction products appeared to be identical to those from thermally catalyzed autooxidations.

Vitamins, with few exceptions, were destroyed by sterilizing doses of radiation; contrariwise, enzymes were not. As a result, a pre-irradiation heat treatment of meat is required to inhibit autolytic softening of the tissue during post-irradiation storage. Beef tissue proteases have been identified and characterized. Most attention has been devoted to a cathepsin which requires ferrous ion for activation. Chemical methods for its control, focusing on control of pH and of ionic strength, are under investigation.

Identification of volatiles stripped from beef during irradiation is nearly complete. Hydrocarbons are the major new compounds found. Alcohols, aldehydes, and ketones, together with hydrocarbons, totaling 41 compounds, have been positively identified; an additional 10-20 are suspected. Differences in amounts rather than in kinds of these compounds appear to be the key to the chemistry of radiation flavor. These differences remain to be determined.

Compounds identified in the volatiles of irradiated beef were evaluated by sensory panels for contribution to the radiation flavor. Of the 29 surveyed initially, 11 were chosen for blending. Thirteen pairs and four blends of three or four compounds had relatively high intensity of radiation odor, although none was identical to that of irradiated beef. These best matches each contained an aldehyde, a nitrogen base, and a sulfur compound.

Probable precursors of the radiation odor in beef have been studied. One compound, first isolated from a phospholipid fraction, may be a glycoprotein. The role of free radical reactions in the productions of odor was studied with inconclusive results.

Studies on odor volatiles, chemical inactivation of enzymes, and the apparent precursor of the radiation odor will be completed in the near future. A new study employing radiofrequency energies to inactivate enzymes without heat will be initiated soon.

Re-evaluation of chemical changes in terms of temperature, dose rate, and total dose during sterilization will be required because of new developments in processing technology and microbiology. The use of newer techniques in texture measurement developed in other Food Division programs will assist in extending knowledge on texture and its change in irradiated foods.

Microbiology (Nicholas Grecz)

Radiation microbiology research during 1961-1963 was primarily concerned with spores of the highly toxigenic and highly radiation resistant organism, *Clostridium botulinum*. The radiation resistance in phosphate buffer of 102 type A and B strains of *C. botulinum* varied from D value of 0.336 Mrad (strain 36A) to D value 0.129 (strain 51B).

The following factors were found to affect radiation resistance of *C. botulinum*: (1) temperature, (2) suspending menstruum, (3) pH (especially at higher temperatures), (4) some chemicals, and (5) some food additives.

Physiological studies indicate that refractibility of spores is related to insoluble phosphates of several metal ions and that viability, resistance to single staining, and phase refractibility can be lost independently under particular experimental conditions.

Massive inocula of heat-shocked spores of *C. botulinum* in experimental food products exhibit (a) residual botulinum toxicity and (b) "tailing" of the survival curves, both of these phenomena are of serious concern to radiation preservation of food and should be further investigated.

An inoculated pack experiment on sliced canned bacon of military ration type was completed in 1962. A statistically safe radiation sterilization dose was calculated to be less than 4.0 Mrad; however, a dose of 2 Mrad will probably be sufficient to produce commercially stable bacon under prevailing food handling conditions.

An inoculated pack experiment with canned chicken is presently in progress.

Acceptance Tests (Elie Weeks)

Large-scale troop evaluations of irradiated foods are conducted by the Quartermaster Research and Engineering Field Evaluation Agency, located at Fort Lee, Virginia.

During 1958 one such test demonstrated that pork loin and bacon, stored at room temperature for 10 to 12 months are equal in acceptability to the equivalent fresh issue pork and bacon. The second 1958 test was less decisive. One meat item, chicken stew, and two pasteurized fruit items, fruit compote and pineapple jam, were equally as well liked as their equivalent unirradiated standards. The other three irradiated test foods were less well liked than their equivalents. They were fried shrimp, fried chicken, and diced carrots.

Following a 4-year suspension, troop testing was resumed in June 1963. Pork loin chops, fried chicken, and bacon were evaluated. It was found that the irradiated pork chops were equally as well liked as the unirradiated standard. However, the chicken and bacon were less well liked.

A second radiated food test was conducted in September and October 1963. The purpose was to determine whether repetitive eating of one meal (chicken), irradiated at 4.5 Mrads, would result in a progressive decrease in ratings when served at the Master Menu frequency and prepared by three recipes. Data obtained during this test is not yet available.

Additional large-scale troop evaluations are scheduled for each quarter through FY 1968 These will include investigations of levels of radiation and time in storage on the acceptability of irradiated foods. Various methods of preparation will also be evaluated.

Packaging (Eugen Wierbicki)

The limited data on the extractives formation by 6.0 Mrad irradiation of polyethylene, polyvinyl chloride, and chlorotrifluoropolyethylene films, when in contact with water, indicate that these plastic films might be suitable for in-package radiation sterilization of neutral foods, such as meats.

The total amount of the extractives produced by irradiation is in the order of magnitude found for thermally treated polyethylene while in contact with distilled water.

The absence of the chloroform soluble extractives in irradiated samples indicate that the dose of 6.0 Mrads didn't cause degradation of the polymers.

The FDA regulations specify that the yield of net chloroform soluble extractives from packaging plastic materials shall not exceed 0.5 milligram per sq in. of the contact area. The four flexible materials investigated have met these requirements.

The research work, now being conducted by the Continental Can Company, will provide a more comprehensive picture on the extractive formation by various food packaging films. Should the result confirm the data available for the four flexible materials, we can look with confidence for the in-package

radiation sterilization of foods, at least from the point of view of their resistance to radiation and the clearance by the Food and Drug Administration for their intended use.

Realizing the type and severity of rough handling, which could be encountered in the military supply system, the development of acceptable flexible containers for the logistic purposes still will take a considerable length of time.

Looking ahead, we plan to have a few prototype flexible containers for radiation sterilized foods at the end of 1966; this will include the necessary clearance by the Food and Drug Administration from the standpoint of extractives.

Study of the performance of these prototype flexible containers with irradiated foods under different environmental conditions and during two years of storage at different temperatures will require additional time before final versions of flexible packages could be used for radiation sterilized foods.

We are optimistic, and our optimism combined with the needed support and "know-how" of the packaging industry will enable us, we believe, after four to five years of additional research and development work, to provide the U. S. Army, and eventually the U. S. public, with wholesome irradiated foods packaged in flexible convenient packaging materials.

Dosimetry (Robert Jarrett)

The dosimetry program for the last two years has been concentrating on obtaining dosimetry systems suitable for use at the U. S. Army Natick Laboratories. After reviewing the literature, the ferrous-copper dosimeter was selected as the working standard, ceric sulfate as the in-line dosimeter, polyvinyl chloride as the go, no-go dosimeter, and ferrous sulfate as the standard by which the other systems are to be calibrated.

After a year of operation, the above systems have proven to be satisfactory for their respective uses. There is, however, need for better systems and more information on the available systems.

Work has been conducted on studying the various dosimetry systems with the more important results being obtained on ceric sulfate.

1. The G value was determined to be 2.32 ± 0.06.

2. The surface effect of the ampules can be expressed by $D = 1.25\, D\frac{A}{V}$.

It was determined that for ferrous systems the surface effect can be expressed by $D = 1.8\, D\frac{A}{V}$.

The areas still to be investigated are:

1. Effects of changing concentration of reagents in making the dosimeters.

2. Calibration of polyvinyl chloride for use with the linear accelerator.

3. The effects of changing the irradiation energy and dose rates on the various dosimeters.

4. Develop and use electrical dosimeters such as ion chambers.

5. Investigate the depth dose distribution using polyvinyl chloride and blue cellophane.

6. Develop an inexpensive go, no-go dosimeter capable of indicating the dose received to ±5 per cent.

Linear Accelerators (Raymond D. Cooper)

The advantages of a linear accelerator as a radiation source for food preservation have been listed, and it has been shown that to a large extent the disadvantages termine the research program in this Laboratory. The first problem associated with the application of the linac is the complexity of the machine itself. Steps are being taken to increase the reliability of operation, the stability of the output beam parameters, and the ease with which the accelerator c n e set up and operated. The second problem inherent in the use of electron sources is the small amount of radioactivity produced in the target at higher energies. The work which has been done on this problem in the past is being re-evaluated, and further studies have been initiated to determine the activity produced as a function of energy. Consideration is being given to the development of possible criteria for defining what is meant by a measurable amount of induced activity. It is hoped that a "highest energy point" can be determined at which electrons can safely be used for a particular food without conflicting with the Delaney additives amendment. The final major problem concerns the measurement and control of the dose rate output of the accelerator and the determination of the way that this dose is distributed in a unity density target. A device has been designed which will continuously calculate and display the surface dose being applied by the electron beam. This will simultaneously measure and control the beam parameters and conveyor speed which determine this surface dose. Investigations have been started which it is hoped will lead to a complete understanding of the interaction of the electrons with the target and thus the distribution of energy throughout the sample. Experimental measurements have been made of charge distributions in various materials as a function of energy, and similar dose distribution measurements will be started within the next few weeks. Together with the dosimetry group, studies are planned of the dose rate dependence of present dosimeters and the

use of other possible dose measuring systems. The technique of cross-firing will be studied to determine the effect of variations in the electron energy and the density of the sample.

Our present knowledge of the application of electron accelerators to food preservation allows us to make an educated guess as to the kind of machine which would be most useful in a production facility. Within a very short time it is hoped that experimental evidence will be available to answer many of the technical and economic questions which still exist.

Conclusion

The national program on Radiation Preservation of Foods has advanced considerably on all major fronts since the Seventh Contractors' Meeting in June 1961. Since that meeting the U. S. Army Radiation Laboratory, Natick, Massachusetts, has become operational and the Marine Products Development Irradiator, Gloucester, Massachusetts, is under construction by the AEC for the U. S. Department of Interior. Irradiated bacon and wheat products have been cleared by the U. S. Food & Drug Administration. Petitions to clear several other foods (potatoes, oranges, lemons) have been submitted to FDA. Within the next three years petitions to clear many of the fruits, vegetables, meats, and marine products will be submitted to FDA. Radiation source technology is keeping pace with food technology. Gamma and electron sources are being simplified, streamlined, improved, made more efficient—all at greatly reduced cost. The last major hurdle is commercialization so that the benefits of irradiated foods can become abundantly available to both military and civilian consumers. I predict that the first irradiated food will be available to the public in three years and that by the end of this decade, irradiated meats, fruits, cereals, and marine products will be commonplace.